Dissertation on

"A STUDY OF FACTORS ASSOCIATED WITH ANEMIA IN HIV INFECTED INDIVIDUALS IN A TERTIARY CARE HOSPITAL"

Submitted in partial fulfillment for the Degree of

M.D GENERAL MEDICINE

BRANCH – I

INSTITUTE OF INTERNAL MEDICINE

MADRAS MEDICAL COLLEGE

THE TAMIL NADU DR. MGR MEDICAL UNIVERSITY

CHENNAI – 600003

APRIL - 2017

CERTIFICATE

This is to certify that the dissertation entitled "**A STUDY OF FACTORS ASSOCIATED WITH ANEMIA IN HIV INFECTED INDIVIDUALS IN A TERTIARY CARE HOSPITAL"** is a bonafide original work done by **Dr.A.SENTHIL** in partial fulfillment of the requirements for M.D.GENERAL MEDICINE BRANCH – I examination of the TamilnaduDr.M.G.R Medical University to be held in April 2017, under my guidance and supervision in 2016.

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I hereby solemnly declare that the dissertation entitled "**A STUDY OF FACTORS ASSOCIATED WITH ANEMIA IN HIV INFECTED INDIVIDUALS IN A TERTIARY CARE HOSPITAL"** is done by me at Institute of Internal Medicine, Madras Medical College & Rajiv Gandhi Government General Hospital, Chennai during 2016 under the guidance and supervision of **Prof. R. SABARATNAVEL M.D.,** This dissertation is submitted to The Tamilnadu Dr. M.G.R Medical University, Chennai towards the partial fulfillment of requirement for the award of M.D. Degree in General Medicine(Branch I).

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ACKNOWLEDGEMENT

I express my heartful gratitude to the Dean, **Prof. Dr. M. K. MURALITHARAN M.S, Mch.**, Madras Medical College & Rajiv Gandhi Government General Hospital, Chennai-3 for permitting me to do this study.

I am deeply indebted to **Prof. Dr. S.MAYALVAHANAN**., Director &Professor of Medicine, Institute of Internal Medicine, Madras Medical College & Rajiv Gandhi Government General Hospital, Chennai-3 for his support and guidance.

I am very grateful to **Prof. Dr. R.SABARATNAVEL M.D.,** Professor of Medicine, Institute of Internal Medicine, Madras Medical College& Rajiv Gandhi Government General Hospital, Chennai-3 who guided and trimmed my work throughout the period of my study.

I am very much thankful for the help rendered by my Assistant Professors and **Dr. J. JACINTH PREETHI M.D.,** and **Dr. K. VIDHYA, M.D.,** for their constant help and encouragement.

I am extremely thankful to all the Members of the **INSTITUTIONAL ETHICAL COMMITTEE** for giving approval for my study.

I express my heartful gratitude to **Dr.M.DEEPA LAKSHMI Dr. R.NAVEEN** and **Dr. A.P.SANKARA AVUDAIYAPPAN** for their constant support and encouragement.

I also thank all the patients who were part of the study and my Professional colleagues for their support and criticisms.

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	PLAGIARISM REPORT	

AIDS was first identified in the United States way back in 1981predominantly in people having monogamous relationship, and Human Immunodefiency Virus was later detected as the causative organism. While starting it is limited, infection with the human immunodeficiency virus increased rapidly over the last half a century to become the most deadliest epidemic in the whole world. According to December 2009, 33.1 million people were approximated to be infected and living with HIV/AIDS, and more than 35.1 million had died from the beginning in the epidemic [1]. Asia is the rapidly increasing in the HIV infected people and majority found in the sub- Saharan africa. Profound immunodeficiency is found in HIV infected individual is hallmark of HIV due to progressive decline in helper T cells.

CD4 cells decline in number below a certain level is high risk for various opportunistic diseases to develop in particularly neoplasia and infection that in AIDS – defining illnesses. Detection of Human Immunodeficiency Virus by tracing its antibodies and also direct detection of Human Immunodeficiency Virus or its components helps for the diagnosis of HIV infection. ELISA is used for screening test for HIV infection and confirmatory by WESTERN BLOT. The best indicator for patients with HIV infected is identified as CD4 + T cells level in laboratory test and to know immunologic competence in same patient. Human immunodeficiency virus found to be etiology for broad spectrum of infectious,neoplastic and immunologic complications. Followed diseases are major concern in HIV course and anemic are also observed may complicate the all condition in HIV patients.

Anemia is rarely a fatal complication, however it does significantly increase the morbidity as well as precepitates preexisting illnesses. Hence these patients found to be reduced survival in high risk Human Immunodeficiency Virus patients. The prevalence of anemia in patients with Human Immunodeficiency Virus varies between 10% in asymptomatic patients to 92% in persons with full blown AIDS[2]. In Human Immunodeficiency Virus patients, anemia is a predictive feature of disease progression or death, independent of CD4 and viral load[2]. Anemia impacts a range of dimensions of quality of life. The common causes of anemia in HIV and non HIV patients are varied so treatment will differ. By knowing knowledge of the association of anemia and HIV infection is important it helps in treatments for anemia are available including r-human erythropoietin, blood transfusion, and in drug causing

anemia, terminating of myelosuppressive therapies. Hence knowledge of the pathophysiological mechanisms and the prevalence of various causes of anemia will help us in treatment of anemia in HIV positive patients. Very few studies have examined factors associated with anemia in the setting of a developing country.

2. AIMS AND OBJECTIVES

AIMS AND OBJECTIVES

1. To study the etiology of anemia in HIV positive individuals admitted in medical ward.
2. To study the relationship between anemia and immunological status as indicated by the CD4 counts

3. REVIEW OF LITERATURE

AIDS was first identified in the United States way back in 1981predominantly in people having monogamous relationship, and Human Immunodefiency Virus was later detected as the causative organism[3, 4]. Around months the disease became clear in male and female individuals, intravenous injection drug addicts and also found in recipients of blood transfusions and hemophiliacs. In 1983 Luc Montagnier isolated HIV from AIDS patient found with lymphadenopathy [5]. In 1985 a precise assay well discovered leading to the early diagnosis and new era of the HIV pandemic worldwide [6]. In the initial days people living with HIV/AIDS (PLHA) had little hope. The introduction of antiretrovirals was a turning point for hundreds of thousands of people. HIV patients have expanded over the past few to become the dangerous epidemic of the last century.

EPIDEMIOLOGY

Incidence and prevalence worldwide [7].

AIDS is a global pandemic with more cases reported from virtually every country. As per the 2015 WHO update the no.of people living with AIDS was around 36 million. This includes 18 million women.

Individuals with AIDS in the year 2015 was around 2.1 (1.8-2.4) million. It was nearly 28% less than the 2.6 million people newly infected in 2009, and greater than 30% less than the estimated 3.million [2.9 million–3.4 million] in 1999, where new infections peaked. The total numbers of AIDS deaths in 2015 were 1.1 (0.94 - 2.1) million. The HIV epidemic happened in waves in various parts of the world, each wave having different features depending on the region's demographics, region in question and timing for introduction of HIV into the common population. The various subtypes of HIV-1 are common in various regions of the world, increasing the more difficult in discovery of vaccines and indicate the various degrees of virulence.

FIGURE 1: PREVALENCE OF HIV INFECTED PEOPLE

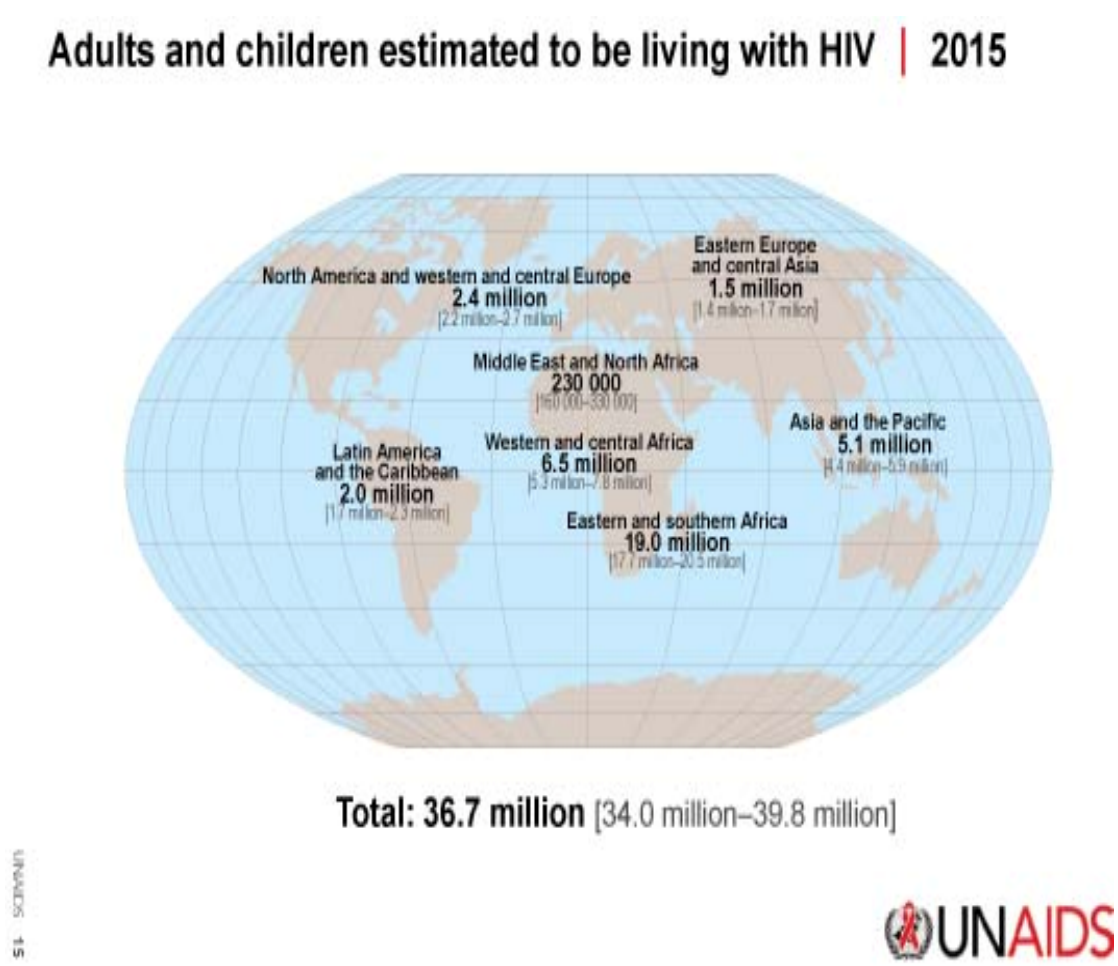


FIGURE 2: INCIDENCE OF ADULT + CHILD DEATHS WITH HIV

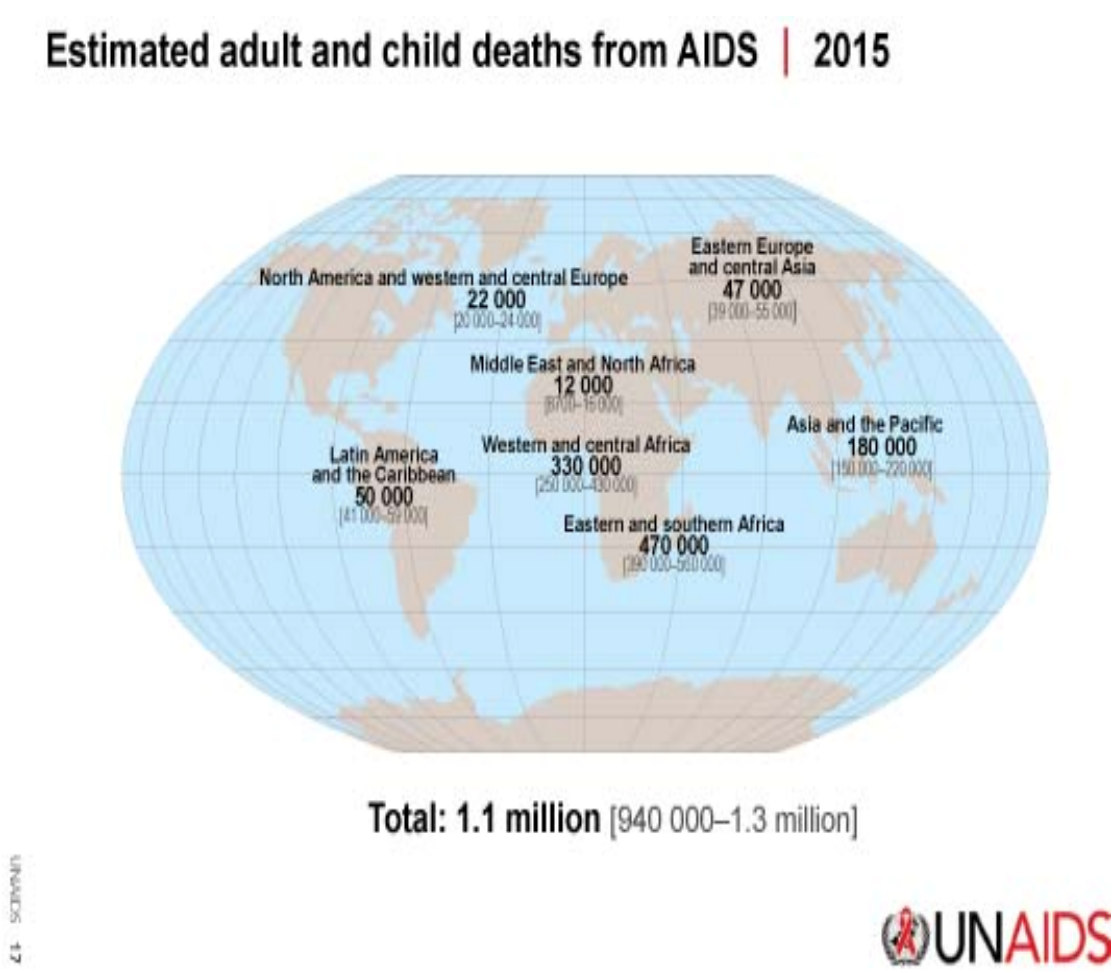
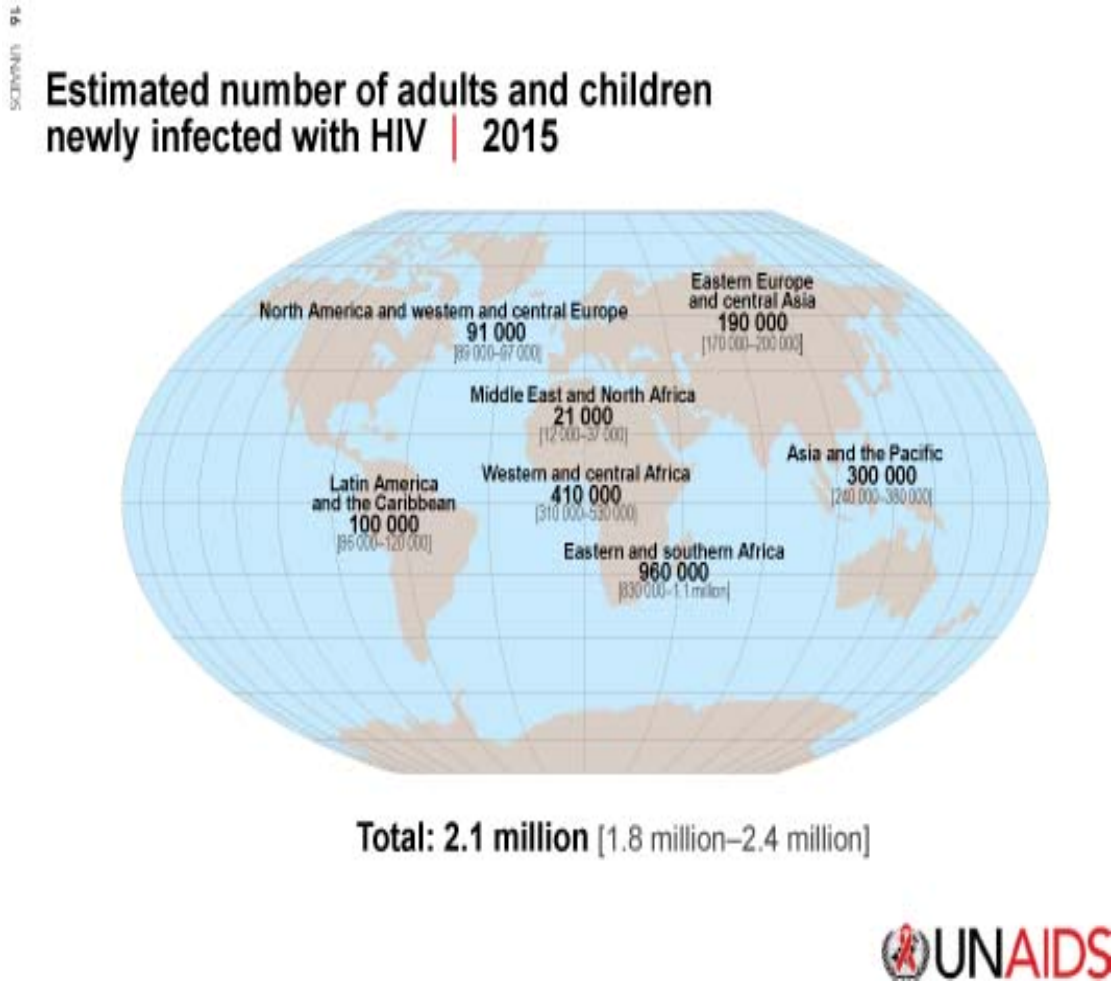


FIGURE 3: INCIDENCE OF ADULTS + CHILDREN WITH NEWLY INFECTED HIV

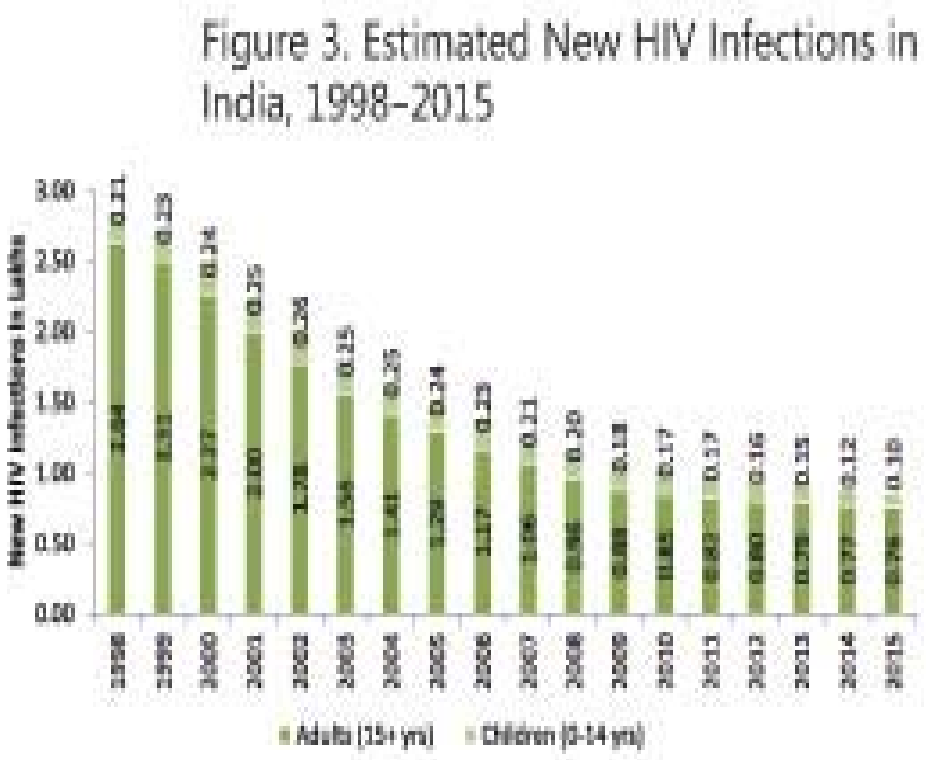


Indian scenario [8 9]

Of the 5.1 million HIV-infected persons found infected in Asia, mostly people in living in India. India had an estimated 2.1(1.71 – 2.64) million HIV infected persons in 2015, with an measured adult HIV prevalence of 0.26% (0.22%–0.32%). There is a decreasing trend of people living with AIDS in the country, from 2.6 million in 2009 to 2.2 million in 2015. Women

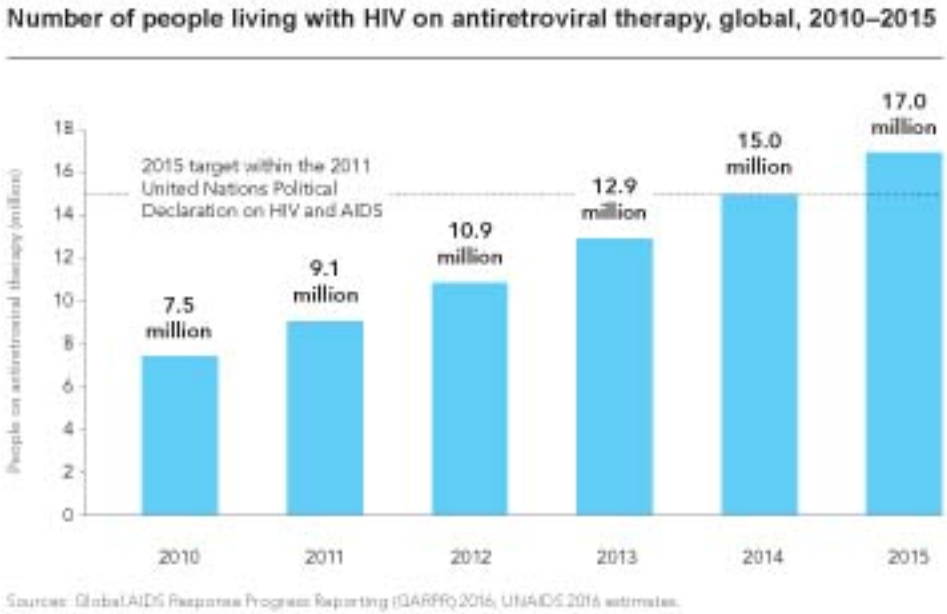
account for 40.5percent of PLHA while children account for 6.54%. Subtype C of M group of HIV -1 is the mostly found prevalent worldwide and in India [10]

FIGURE 4: PREVALENCE OF NEW HIV INFECTED PEOPLE IN INDIA



CLINICAL MANIFESTATIONS

FIGURE 5: NUMBER OF PEOPLE WITH HIV INFECTED ON ART



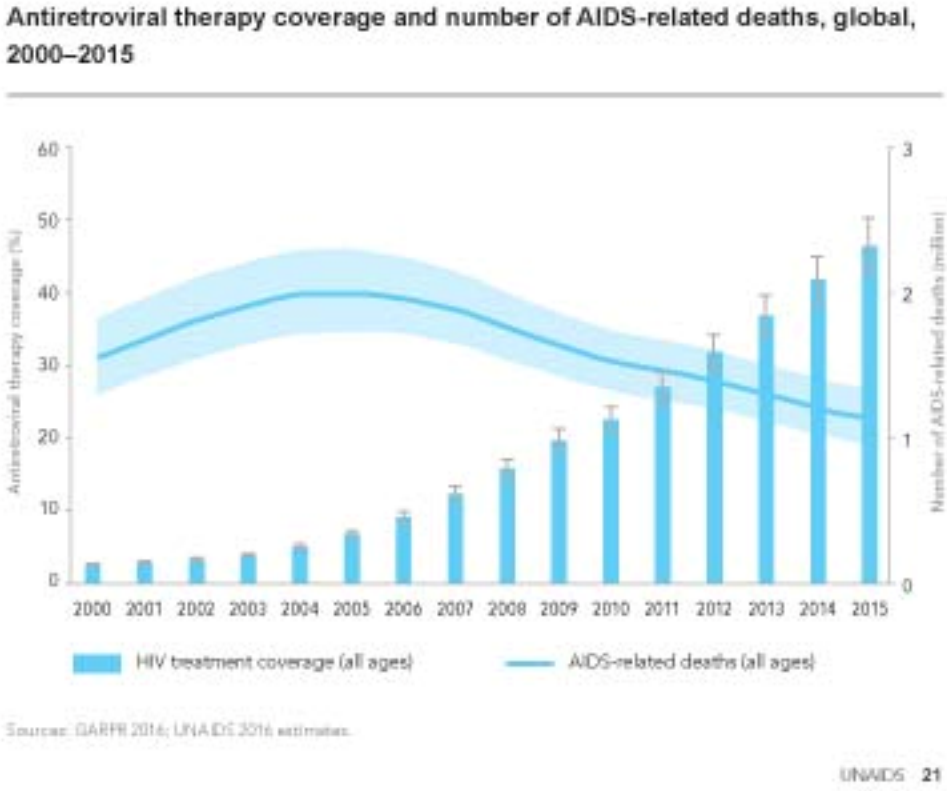


FIGURE 6: DISTRIBUTION WITH PLHIV IN DIFFERENT STATES IN INDIA

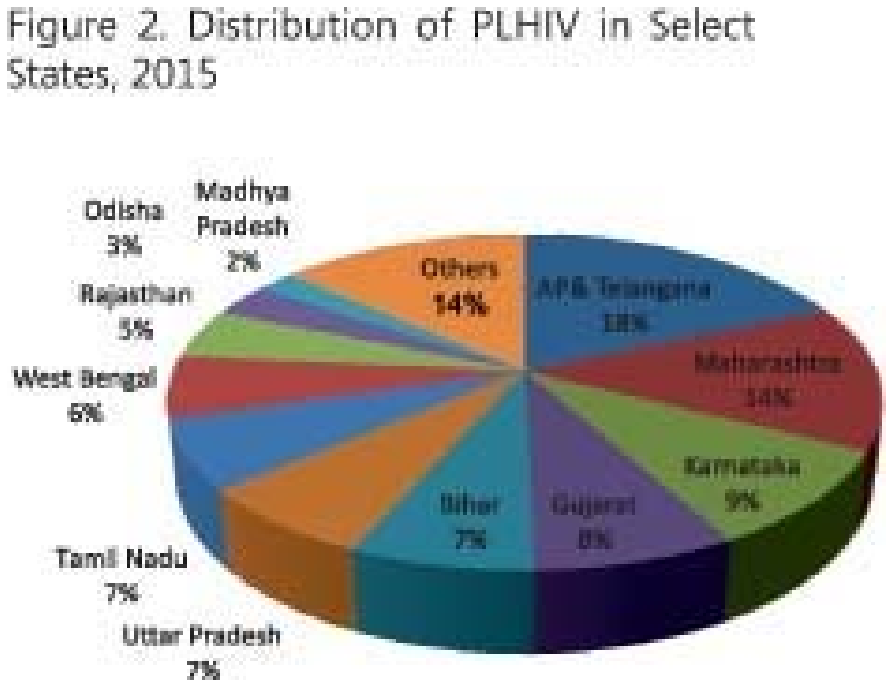
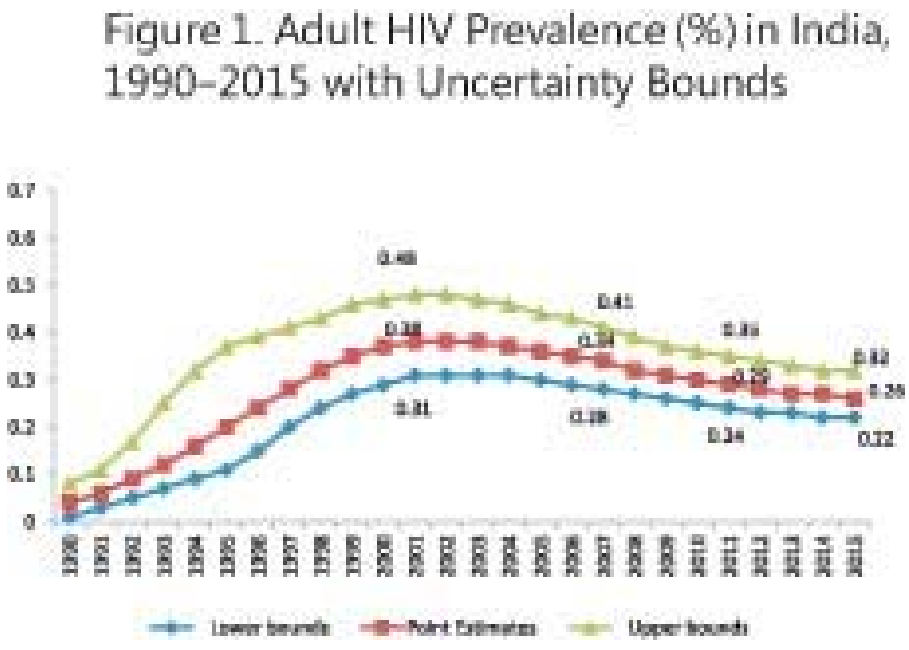


FIGURE 7: DISTRIBUTION WITH PLHIV IN DIFFERENT STATES IN INDIA



Acute HIV Syndrome

Acute HIV syndrome occurs 3-6 weeks after infection in 50-70% of infected individuals and can vary in severity from individual to individual. It presents as an acute mononucleosis like illness. Symptoms last one to several weeks

Asymptomatic Stage/Period of Clinical latency

The progression to clinical disease occurs in about10 years. Patients are usually asymptomatic during this period or may continue to have persistent generalized lymphadenopathy. Dermatological manifestations may occur.

Symptomatic/Intermediate Stage

This is the stage when the CD4 count is between 200/µl to 500/µl. Usual complications at this stage include dermatological, oral and constitutional manifestations.

Stage of AIDS

The CD4+ count is lower than 200permicrolitre in these patients. The opportunistic infections, neoplasms and neurological disorders seen at this stage constitute the AIDS defining conditions

Centre for Disease Control classification system for patients with HIV infection in adults and adolescents [10]

The latest Centre for Disease Control classification in patients with HIV infection in adults and adolescents classifies persons with the underlying of clinical conditions along with HIV infected and CD4+ T lymphocyte counts. The classification differentiates three ranges of CD4+ T lymphocyte [11]

Counts and there are three clinical categories also represent with nine mutually exclusive categories

Table -1

CDC Classification System for HIV-Infected Adults and Adolescents

CD4 Cell Count Categories	Clinical Categories		
	A Asymptomatic, Acute HIV, or PGL	**B** Symptomatic Conditions, not A or C	**C** AIDS-Indicator Conditions
(1) ≥500 cells/µL	A1	B1	C1
(2) 200-499 cells/µL	A2	B2	C2
(3) <200 cells/µL	A3	B3	C3

CATEGORY A

Asymptomatic in HIV infected individual

Prolonged generalized lymphadenopathy

Acute HIV infection also accompanying illness or history of acute HIV infection

CATEGORY B

Bacillary angiomatosis

Candidiasis – oral,pharyngeal,vulvovaginal

Cervical dysplasia

Constitutional symptoms as fever >38.5 c

Diarrhoea >1 month

Hairy leukoplakia

Herpes zoster

Idiopathic thrombocytopenic purpura

Listeriosis

PID

Peripheral neuropathy

CATEGORY C

Candidiasis – trachea,bronchi,lungs,esophageal

Cervical cancer

Coccidiodomycosis

Cryptoccosis –extrapulmonary

Cryptosporidiosis >1 month

CMV Diseases

Cmv retinitis

Encephalopathy

Herpes simplex > 1 months duration

Histoplasmosis

Isosporiasis

Kaposi sarcoma

Lymphoma –burkitt,brain

MAC- Extrapulmonary

Mycobacterium tuberculosis

WHO staging system for HIV infection in adults and adolescents [11]

Clinical stage 1

- Asymptomatic
- Persistent generalized lymphadenopathy
- Unexplained moderate weight loss (<10% of presumed or measured body weight)
- Recurrent respiratory tract infections (sinusitis, tonsillitis, otitis media, pharyngitis)
- Herpes zoster

Clinical stage 2

- Angular cheilitis in hiv infected individual
- Recurrent oral ulceration
- Papular pruritic eruptions
- Seborrhoeic dermatitis
- Fungal nail infections
- Cant explained more weight loss (>10% of presumed or observed body weight)

- Cant explained chronic loose stools for more than one month
- Cant explained persistent fever (above 37.5oC on &off or constant for more than one month)
- Persistent oral Candidiasis in hiv infected individual

Clinical stage 3

- Oral hairy leukoplakia
- Pulmonary tuberculosis
- Severe bacterial infections (e.g. pneumonia, empyema, pyomyositis, bone or joint
- infection meningitis , bacteraemia)
- Acute necrotizing ulcerative stomatitis, gingivitis or periodontitis
- Unexplained anemia (<8 g/dl), neutropenia (<0.5 X 109/litre) and or chronic
- thrombocytopenia (<50 X 109/litre3)
- HIV wasting syndrome
- Pneumocystis pneumonia
- Recurrent severe bacterial pneumonia
- Chronic herpes simplex infection (or labial, genital or anorectic of more than one month's
- duration or visceral at any site)

- Esophageal candidiasis (or candidiasis of trachea, bronchi or lungs)
- Extrapulmonary tuberculosis
- Kaposi sarcoma
- Cytomegalovirus infection (retinitis or infection of other organs)
- Central nervous system toxoplasmosis
- HIV encephalopathy
- Extrapulmonary cryptococcosis including meningitis
- Disseminated non-tuberculosis mycobacterium infection

Clinical stage 4

- Progressive multifocal leukoencephalopathy
- Long term cryptosporidiosis
- Long term Isosporiasis
- Disseminated mycosis (extrapulmonary histoplasmosis, coccidiomycosis)
- Repeated septicaemia (including non-typhoidal - salmonella)
- Lymphoma (B cell non-Hodgkin/ cerebral)
- Invasive cervical carcinoma
- Atypical disseminated leishmaniasis
- Symptomatic HIV-associated nephropathy or symptomatic HIV-associated cardiomyopathy

Hematologic manifestations in HIV infection

Anemia,leukopenia,lymphadenopathy, and thrombocytopenia are common disorders in hematapoietic system throughout all course of HIV infected individual and found it was a result of AIDS, shows features of 2^0 malignancies and infections and some adverse effects of treatment. Most of patients, or else asymptomatic, also develop persistent whole lymphadenopathy of early clinical manifestation in HIV infected person. This conditions are verified by the presence of lymphadenopathy in greater extrainguinal sites for more greater than 2 months without an clearly seen etioligy. The enlarged lymph nodes is because of the prominent follicular enlargement in infected node due to AIDS. Infected cases with CD4+ T cell counts greater than 200per microliter, the observed diagnosis of lymphadenopathy also consists of Kaposi sarcoma, Tb, Castleman's disease, and lymphoma. Infected cases with advanced infection, lymphadenopathy can be found due to atypical mycobacteria, systemic fungi, and or bacillary angiomatosis.

TREATMENT[12]

Initiation of ART by level on CD4 count and developed by WHO clinical staging:

CD4 count available

– Stage IV: Irrespective of CD4 count

– Stage III: CD4 count < 350/µl

– Stage II & I: CD4 count < 200/µl

CD4 count not available

– Stage IV & III: Treat

– Stage II & I : Do not treat

Recommended choice of first line regimens:

Choose lamivudine in all regimens; Choose one NRTI to combine with lamivudine; Choose one NNRTI

Preferred first line regimen : AZT + 3TC +NVP

Alternate 1st line regimens :

AZT + 3TC + EFV

D4T+ 3TC + (NVP or EFV)

ANEMIA

Anemia is identified by a decline in Hb or Hct value in an persons normal value. Because of person normal Hb levels mostly are not well appreciated, specific for sex and race determine the reference ranges are always used for the working diagnosis in anemia. In general population, normal Hb values are 1 to 2 g/dL lower in female and also in African American male and than in white male. WHO criteria used for anemia to diagnose in men and women are <13 & <12 g/dL, respectively [13]. These criteria are meant and to be used around within the context of international nutritional studies, and are not initially designed to used as gold standards to the diagnosis of anemia.

Normal ranges used for population with in a higher incidence of chronic disease may also be skewed following anemic levels. Thus, anemia is very difficult to diagnosis in countries in which malnutrition,added infection (example : HIV infection/ tuberculosis) and/or congenital related hematologic disorders are common.

Clinical consequences

Grades of anemia is used for to asses signs/symptoms of anemia related and also the rate at which it has evolved, along with the oxygen demands of infected patient. Symptoms are observed as much less with anemia that shows slowly, because there is time for various factors influence to multiple homeostatic forces to make adjust for reduced oxygen carrying capacity of blood.

CLASSIFICATIONS OF ANEMIA

Physiologic classification

Disorders resulting in an inability to adequately produce red blood cells . Disorders resulting in rapid RBC destruction (hemolysis) or RBC losses from the body

Etiology of the anemia is very well evaluated with the reticulocyte count. With increased reticulocyte count mostly is seen as normal bone marrow response for ongoing hemolysis / non continuous blood loss. On the other side, low reticulocyte count, which shows decreased production of erythrocytic cells,and is more consistent to show with bone marrow depression.

These two categories are not mutually exclusive.

Morphologic classification

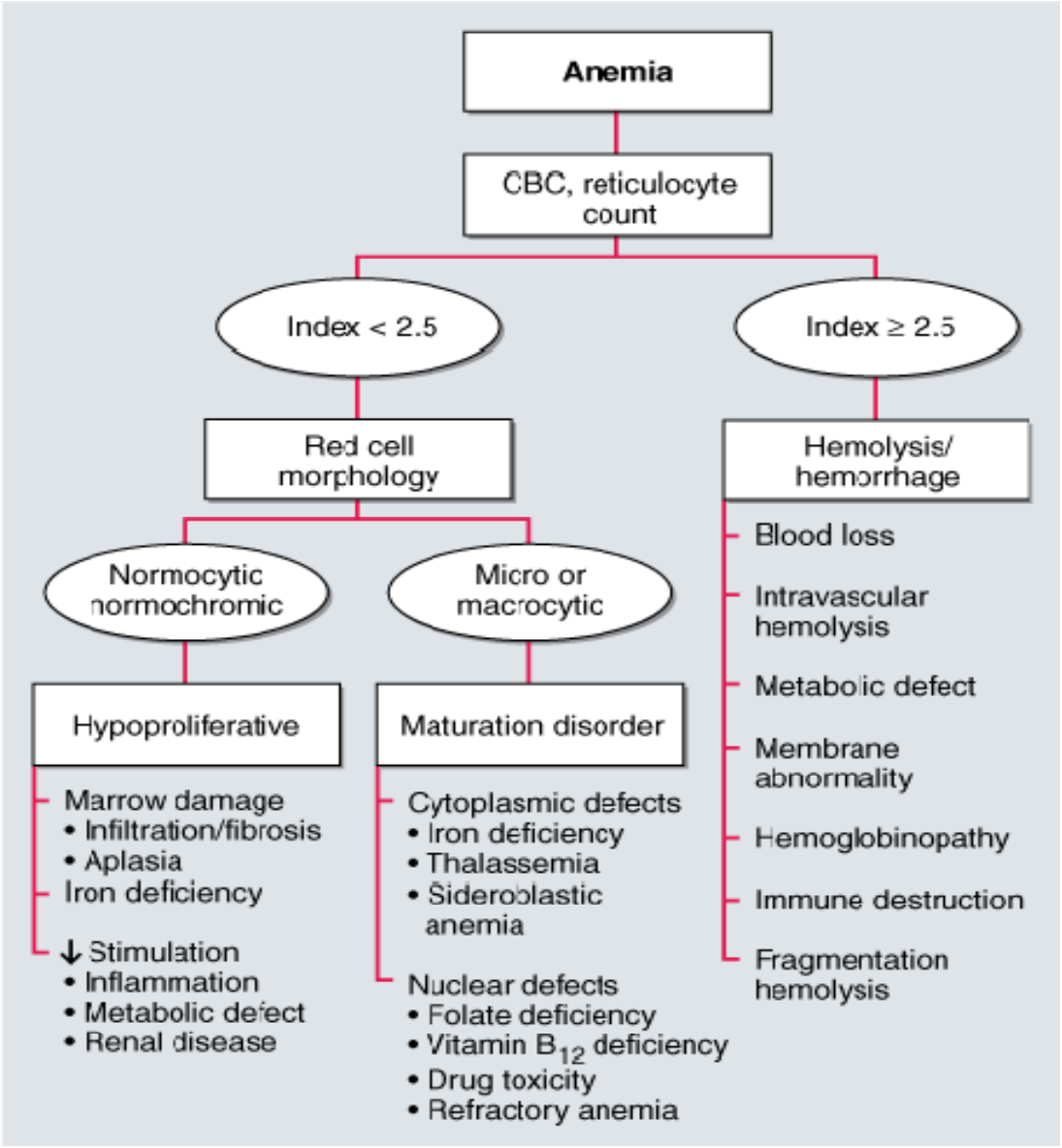
- Normocytic
- Microcytic
- Macrocytic

(WHO)/AIDS Clinical trial group (ACTG) criteria of anemia grades for both men and women are as follows [29]:

- Grade one 9.5–10.9 g/dl
- Grade two 8–9.4 g/dl
- Grade three 6.5–7.9 g/dL
- Grade four < 6.5 g/dL

The following figure shows the physiologic classification of anemia

FIGURE 8: PHYSIOLOGIC CLASSIFICATION OF ANEMIA[10]



ANEMIA IN HIV INFECTION

Anemia in HIV infected patients with incidence reflect varies from 10 - 12% is with no symptomatic patients up to 90 - 93% persons with fully infected AIDS [2]. Most common hematologic variation is seen is anemia associated with HIV infected [15]. Eventhough it cause very rarely a death leading problems of HIV disease but anemia may moderately increase death rate in HIV infected patients.[16] [17] The presence of anemia is always related to increased morbidity and mortality in both children and adults with HIV infection [18] -

Anemia had risk factors to developed were evaluated in a brief manner from the HIV Study (WIHS) & four mojor factors are identified: Mean corpuscular volume (MCV) <80 fL, HIV-1 viral load ≥50,000/mL,CD4 count <200/microL and use of AZT in past six months[16]. Inspite of development of HAART, HIV-caused anemia is more common, and independently accompany risk for decreased survival [21] [22]

Possible role of HIV

HIV as single factor leads to be a major contributor to show variables in hematopoeisis. HIV infected has been very clearly demonstrated in CD4+ lymphocytes, monocytes, macrophages, and dendritic cells [23.] In comparison, diseases of CD34+ bone marrow progenitor cells remains controversial. The in vitro ability of HIV to infect CD34+ cells from healthy donors has been suggested by finding viral inclusions on electron microscopy [24] and demonstrated by nucleic acid amplification [25] . However, most investigators have been unable to detect [26] or have detected only small amounts of HIV-derived DNA [27] from CD34+ progenitor cells isolated from HIV-positive patients. Furthermore, the most primitive subset of CD34+ cells appear to be increased in resistant to HIV people, despite HIV receptor expression.

According to kreuzer et al[29] potential pathogenetic etiology of anemia in HIV positive patients are HIV infection of hematopoietic stem cells or red cell progenitor Bone marrow diseases like (Mycobacterium tuberculosis and atypical variant) Bone marrow malignancies like (NHL, HL and Kaposi's sarcoma)

- Myelosuppressive drugs like (Zidovudine , Ganciclovir)
- Vitamin B12, serum folate level, iron related deficiency (undernourised and under absorption, disturbed usage)
- Autoimmune RBC hemolysis like (erythrocyte cell auto antibodies, and flowing immune complexes)
- Loss of Blood due to (Internal bleeding)

PERIPHERAL BLOOD AND BONE MARROW

Most common variant of anemia is mostly normocytic normochromic. Erythrocyte cell morphology is more time observed as normal but abnormalities such as poikilocytosis,rouleaux formation and other formations have been reported[30]. Factors unrelated to HIV infection also cause microcytosis and macrocytosis. Macrocytic anemia is closely observed and reported very frequently in patients on HAART. Most favour cause of macrocytic anemia in HIV infected patients is 2^{o} to HAART[29]. In bone marrow a different types of morphologic characteristics have been described, ranging from normal hematopoeisis to bone marrow dysplasia, plasmacytosis, reticulin fibrosis, lymphocytic infiltration, granulomatous myelitis, and other conditions[32]. The mechanism of HIV related to induced bone marrow changes and peripheral blood changes are observed as a either directly related effect of HIV, nutritional related deficiencies,secondary related frequent opportunistic infections or bone marrow related suppression by drugs and other involved factors. In one study done by Tripathi et al in Lucknow,India, bone marrow examination was normocellular in 76%, hypocellular in 7% and hypercellular in 16% patients[32]. In another study done by Xiaohui in 32% had hypocellular, 32% had hypercellular marrow, 35% had hypercellular marrow [33]. Bone marrow varities are seen in mostly of all stages of disease, and increasing in the thing as the disease going on. Infection like marrow mesenchymal stem cells also been observed as the main factor causing bone marrow defects and all[34]. Intense vacuolization specially in the granulocyte element is frequent. Irregularerythropoiesis may also manifest as more megaloblastic

change. This changes was found not related to serum cobalamin and serum folate levels or to other drug therapy with zidovudine related or folate antagonists related, although these all drugs also may accentuate it [35]. According to Pardela et al[36] 90%of patients of patients are very strongly showed to be have bone marrow irregularity during the full course of disease

ETIOLOGY OF ANEMIA

ANEMIA OF CHRONIC DISEASE

Anemia due to chronic disease is found as the most related favour cause of anemia in HIV-infected patients. Anemia due to chronic disease is explained as anemia mostly not able to be explained by detailed mechanisms and is special thing as by low level of ferremia in the picture of With enough fe stores with normal or high ferritin. Red cells are mostly commonly seen as normocytic and normochromic; there may be with mild or moderate anisocytosis with a few microcytes[37].

Most of the mechanisms are

Direct correlate toxic effect of human immune deficiency virus infection on hematopoietic stem cells / bone marrow

Irregular habit of inhibitory cytokines.

Decrease level of erythropoietin

Defective mechanism in iron metabolism & reused.

HIV may have direct toxic effect on the bone marrow. According to Means et al bone marrow stromal cells also mostly be infected with HIV[38]. Eythroid precursors (glycophorin A positive) can also express CD4 antigen related and thus can be infected with Human immunodeficiency virus[39] . There is directly related cytotoxic effect of HIV seen on various hematopoietic stem cells and as well also on stromal environments of the bone marrow. This results to decline in production of IL-3 and G-CSF leads to defective erythropoiesis [40].

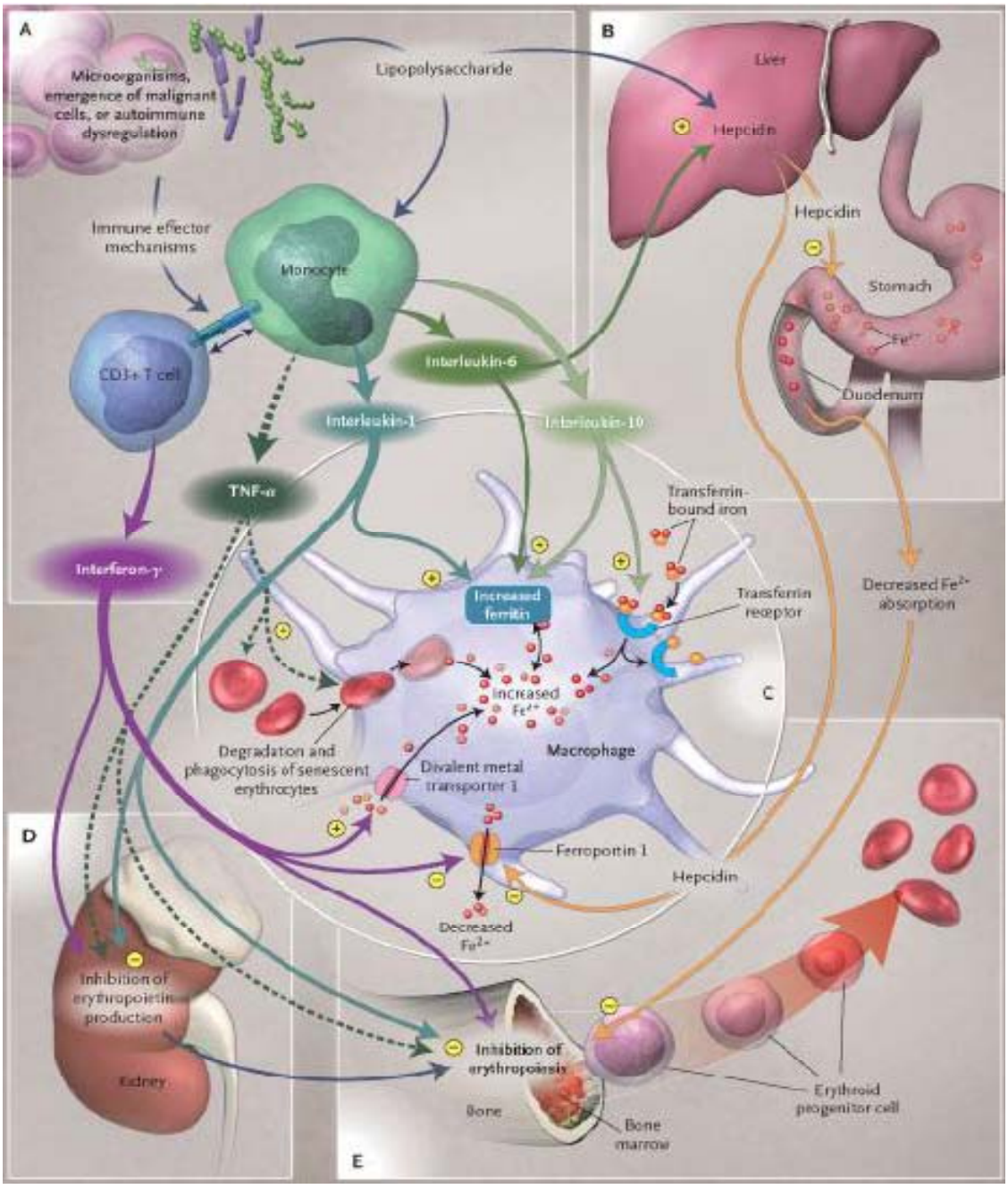
Abnormal expression of the cytokines it affects the most of bone marrow. The bone marrow and its micro environment has a major role in hematopoeisis and is affected by both cellular and humoral factors. All like T cell receptor (gamma delta T cell receptor positive lymphocytes) cytokine induced signaling also decline progenitor growth in vitro [41]. Interferon gamma has also shown to stop growth action of CFU-E through straight receptor binding [42]. Transforming growth factor beta can also suppress progenitor growth in vitro[43]. Study by Fuchs et al has shown that inhibitory effect TNF-alpha, IL-1, and IFN-gamma on bone marrow erythropoiesis contributes to marrow hypoplasia.

Decreased erythropoietin production and decreased responsiveness of stem cells to erythropoietin has been postulated as a mechanism of anemia in HIV positive patients [44.] All patients compared with routine iron deficiency anemia,found to be inadequately less level of erythropoietin again and again reported in HIV infected patients [45] [46]. In treatment as additional with r-erythropoietin had a particular significant effect in large number of anemic human immunodeficiency virus individuals [47] [48] . This made to the hypothesis that says HIV induced anemia may be at less likely due to reported hypoerythropoeitinemia. Pro inflammatory cytokines, TNF alpha & IL-1 beta may happen to less renal erythropoietin synthesis.

Inflammation induced anemia is hall mark to the development of disturbances in normal Fe homeostasis, with incline in retention and uptake of Fe around cells of RE system. The immune effector mechanisms produce cytokines like TNF alpha, IL-1, interferon gamma, ,IL-6 and IL-10. This stimulates the hepatic activity in acute phase protein hepcidin , this expression stops duodenal uptake of Fe. Interferon gamma it increases the action of divalent metal transporter-1 on macrophages & stimulates the absorption of Fe^{+} iron. It also may decrease the action of macrophage iron transporter Ferroportin-1, and stop iron take up in macrophages,and a way that is involved and involved by hepcidin [49]. Ferritin concentrations incline with Human immunodeficiency virus disease process, and Fe settles in most tissues. Fe excess may also shows negative effects because of an Fe-mediated oxidative stress in patients with Humanimmunodeficiencyvirus infection. Iron-mediated oxidative stress may contribute to viral cytopathogenicity and iron storage seems to be associated with shorter survival[29].

The various factors involved in anemia of chronic disease are depicted below:

FIGURE 9: FACTORS IN ANEMIA OF CHRONIC DISEASE



HIV-RELATED INFECTIONS

Bone marrow in HIV patients are the most likely bacterial pathogen to infect with atypical mycobacteria. Erythroid hypoplasia was happen to be a distinctive feature associated with mycobacterun avium intracellulare (MAI) infection. In the study by Gardener et al says bone marrow provided a high yield for microbiological culture, particularly in MAI infection [50] . In the study by Fuller et al says most infectious leads of anemia in HIV- patients due to bone marrow infection include tuberculosis, histoplasmosis, parvovirus B19 , cryptococcosis, and pneumocystis jirovecii and toxoplasma gondii apart from MAI [51]. Opportunistic infections in the gastrointestinal tract including microsporidia are most likely to involved in mechanism of anemia by major cause to Fe, vitamin B12 and related folate underabsorption [52]. In a prospective study by David et al done in 106 adults admitted consecutively to medical wards with hemoglobin <6.9g/dl, patients were worked up for possible bacterial, mycobacterial, parasitic and nutritional related causes of anemia, which includes bone marrow aspiration.One-third of hiv infected had tuberculosis, which diagnosed mainly by bone marrow culture in 8-9% of HIV-infected patients [53].

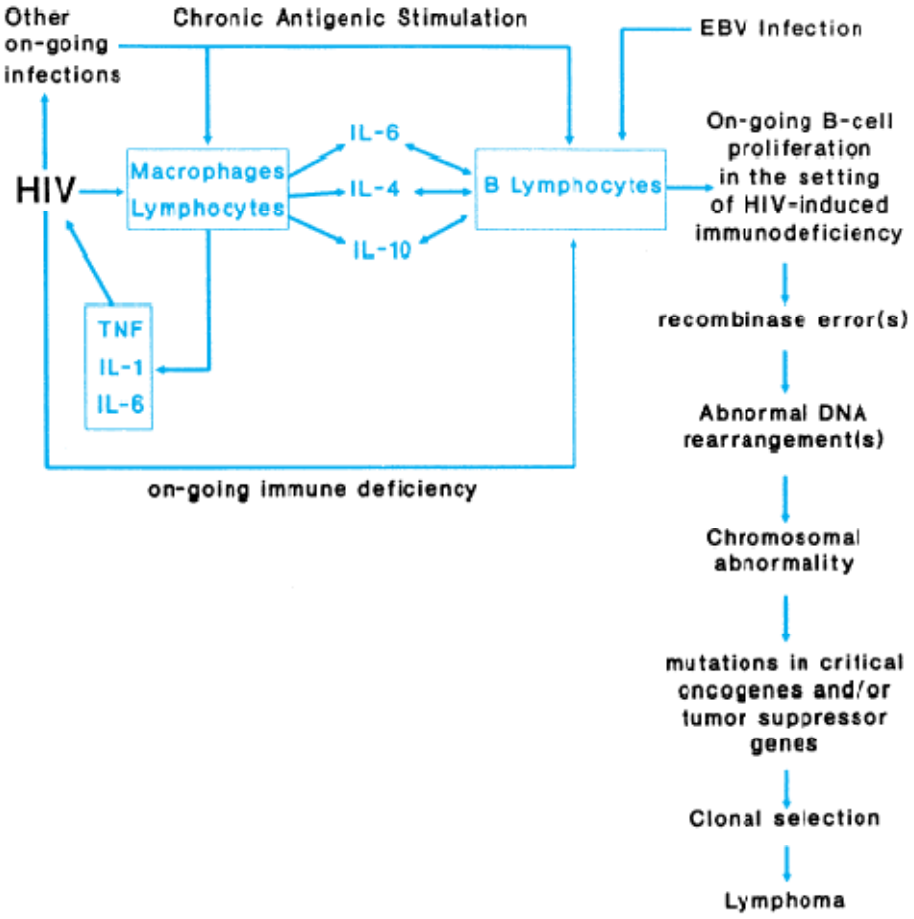
These studies done in the western population show various pathogenic organisms comparing to the Indian population. In India the most common pathogenic organism causing bone marrow infiltration is most likely mycobacterium tuberculosis. It is observed that TB is 2nd common opportunistic diseases in India (next to oral candidiasis), may also greatly increase the load of anemia . The etiology of anemia in TB is multifactor involvement, leads from a collectively of anemia of chronic disease, bone marrow infiltration and leads to various lag of nutrients such as selenium, Fe,vitamin and selenium [54]

MALIGNANCIES

Malignancies also cause anemia by direct bone marrow infiltration and by indirect means. Bone marrow infiltration of (NHL) ,Kaposi's sarcoma, & Hodgkin's lymphoma are described. The development of lymphoma the setting of Humanimmunodeficiency infection the mechanisms underlying fully they are not readed. 1 main involvement may due to immune suppression as own,which on go inline along an incline of lymphoma as in particular congenital immunodeficiency infection, related autoimmune disorders or in using as immunosuppressive drugs. The lymphomas in hiv infected people develop in these settings are same in terms of the pathological grade, the increase level of extra nodal infection reflects presentation, & relatively bad outcome. HIV people is associated with a myriad of immunologic deficiency. These abnormalities added chronic involvement of B lymphocytes antigens, mitogens, or viruses, and to Epstein-Barr virus (EBV), functional & quantitative defects of CD4 T cells and HIV itself. B cell action and related activation lead to the development of reactive B cell overactivity in all lymphoid tissues and also polyclonal hypergammaglobulinemia in hiv infected blood. Lymphomas origin from involvement of genetic errors while happening during the period of polyclonal B cell multiplication in the reason of shown immunodeficiency. This finding has been observed in a first experiment, in that high-grade B cell lymphoma observed between five and fifteen months after hiv diseases with the simian immune deficiency virus, coincident with development of severe immunodeficiency[55]

In one study says that linked AIDS and cancer entries in particular parts in USA, the relative risk to develops lymphoma within three years of an HIV finding was increased by 167-times with compared to people HIV negative. The same study again clearly explains that the high in risk varies from 654-times for high-grade diffuse immunoblastic tumors to 262-times to Burkitt lymphomas, 114-times for intermediate-grade lymphomas, and 14-times for low-grade lymphomas[56]

FIGURE 10: SEQUENCE OF EVENTS IN LYMPHOMA IN HIV INFECTION



Although use of HAART happen to show result to a major and dramatic decline in the new cases of Kaposi sarcoma, results from studies evaluating the effect of HAART on the new cases of lymphoma have inconclusive. The Swiss Humanimmunodeficiencyvirus Cohort study & Multicenter AIDS Cohort Study result says no decrease in the new cases rates of lymphoma within the pre-HAART & HAART eras, but the International Collaboration on Humanimmunodeficiencyvirus and Cancer and the EuroSIDA studies clearly says with valid decline of lymphoma in the HAART era[58] . Data from a French study says that who all taking HAART is most effective in population the new cases of lymphoma will decline [57 58]. Taken together, these data suggest that the incidence of AIDS-related lymphoma in a population depends on the effectiveness of HAART is studied with increasing the immune stage of that paticular people. If HAART is not same type is available or if

HAART is ineffective in increasing CD4 counts or decreasing HIV RNA levels, in this condition the incidence of lymphoma in that population will not be decline.

DRUGS.

More than 22% of all cases of anemia related with Humanimmunodeficiencyvirus are may be drug causing [60]. Recognizing that it as the specialised side effect is mostly problematic by on going disease. Multidrug regimens always alter things difficult to find reason for anemia and also single drug may be the main source of anemia and often have an synergistic fedback on bone marrow suppression.

The commonest agents all we used are zidovudine (AZT) & trimethoprim sulphamethoxazole. AZT causes most certain macrocytosis , which may presently caused by many as screen to patient's treatment with therapy. Bone marrow with hypoproliferation & also dysplasia are seen frequently. Both AZT and d4T cause macrocytosis, AZT, most myelosuppressive effects seen both in vitro and in vivo[29]. Anemia treatment includes with AZT which also is used in the treatment pneumocystis carinii diseases in HIV is because of folate related deficiency & is more obvious over patients associated poor nutritional thing. Dapsone also with the another option was happens to generalized myelosuppression also causes hemolytic anemia

Drugs commonly causing myelosuppression in HIV positive patients[59]

Anti viral

- Zidovudine
- Gancyclovir
- Zalcitabine
- Foscarnet
- Cidofovir

Antifungal

- Flucytosine
- Amphotericin

Anti Pneumocystis drugs

- Sulphonamides
- Pyrimethamine
- Trimethoprim,
- Pentamidine

Antineoplastic drugs

- Cyclophosphamide
- Doxorubicin
- Paclitaxel

Immune response modifiers

- Interferon alpha

Zidovudine and anemia

Anemia in AZT reflects as the dose dependence and duration associated and is frequently accompanied with macrocytosis during bone marrow depression due to use of zidovudine is infrequent[61] . Inhibition of related thymidine monophosphate phosphorylation due to zidovudine monophosphate happens to intracellular thymidine triphosphate low level and stem cell observation also be observed due to the additictive of thymidine. AZT treatment suppresses the proliferation of cells of erythroid, granulocyte/macrophage & primary haematopoietic stem cell cultures of human bone marrow also causing hypoplasia. Various reasons

have been postulated for the hematological effects on zidovudine. Zidovudine may decrease the level of EPO receptor expression & also function on erythroid hematopoietic cells.. AZT particularly inhibits beta globin gene expression to human erythroid precursors happening to clearly seen cell growth inhibition. A toxic metabolite 3- amino 3-deoxythymidine has well noticed in cultured hepatocytes & microsomes which is mostly toxic to BMC specialy erythroid lines[62]. Mitochondrial toxicity found to be suggested to take part as incubation action of the murine erythroleukemic cell line of AZT happens to decrease in action of cell growth & inhibition of oxidative phosphorylation[29] .

Use of HAART was found with an incline in hemoglobin levels 1 year of follow-up (42%) in a prospective cohort study done in Johns Hopkins university. Infected Patients who are not receive HAART theraphy had a known modest increase(31%) in hemoglobin level observed over 1 year of regular follow-up. When used part of a HAART regimen treatment,infected patients who are all receiving zidovudine found to be hemoglobin response that was not observed most significantly varies from patients who also had alternate HAART regimens [70] In one systematic review (6 RCTs, 1597 people), which found that, over 48 weeks, zidovudine related with a hemoglobin drop while hemoglobin rise in people taking stavudine. In all of the RCTs 64 the number of grade 3 or 4 episode of anemia and neutropenia was very high with zidovudine when compare with stavudine .Then with available witness comparing zidovudine vs stavudine comes from resource rich settings. Strength of the results about equal benefits and harms are seen,its applicability to see poor settings is limited by the fact that the RCTs included more men and fewer other ethnic background than seen in many resource poor settings.

In a study done in Varanasi India in which patients were followed up over a period of one year 16 hiv patients found ZDV induced anemia[63]. This comparison found to be very higher to other reports where the amount of anemia varies between 5.6 and 9.9per cent (cut off were 7 and 7.5 g% respectively)[65] .All Peripheral smear done for those patients showed normocytic, normochromic picture in 44 per cent patients and also others featured macrocytic changes. In bone marrow examination dysplastic variables were seen in 8 (30%) patients in myeloid line and 7 (28%) patients showed dysplastic changes in erythroid line.overall most of the

patients with anemia diagnosed within six months after initiation of therapy was resulted in other studies 66. Two patterns were also noted in the causative of anemia: (i) progressive fall in haemoglobin in around 60% of patients, and (ii) an abrupt fall in haemoglobin in around 40% of patients. In majority of the patients (77%), there is well recovery in hemoglobin levels once they stopping zidovudine as soon as possible. Eight patients developed anemia after 1 year and in 4 patients recovery took as long as 7 months. This process very well explained by the factor that myelotoxic effect of drug zidovudine mostly can persist as long. Risk factors foun with zidovudine related anemia reported in various studies supported advanced HIV disease, low body weight, low CD4 counts and low baseline haemoglobin[67]. .There has also been also been case reports of zidovudine causing pure red cell aplasia, which was fully reversible[68]. In a prospective cohort study done in rural Cambodia the odds of developing anemia within 12 months after the switching from stavudine to zidovudine were high in patients mostly with CD4 count < 200/mm3 point at the time of ZDV starting, in malnourished patients, and in same patients also on co-trimoxazole prophylaxis while starting ZDV initiations 69.

Nowadays mean zidovudine dosages are much less than those used in 1980s. According to this study ZDV related anemia has decreasing trend of less likely and so comparable Hb values are also seen in both ZDV treated and non ZDV treated Humanimmunodeficiencyvirus patients [71] Most drugs are identified of increasing ZDV plasma concentrations that also cause & ZDV toxicity increased. Fluconazole and atavaquone directly decrease ZDV clearance. Chloramphenicol, Indomethacin, Naproxen, Ethinylestradiol, Acetylsalicylic acid, Oxazepam and also probenecid can stop liver clearance of zidovudine Furthermore lamivudine also increases zidovudine concentration .Liver cirrhosis which is correlate seen in patients with HIV/Hepatitis B Virus or HIV/Hepatitis C Virus co infection very deeply involved in zidovudine metabolism

In mice bone marrow, heme exerted a very good protective effect on hematopoietic progenitors in vivo and be of very important clinical use in combination with Epo to promote relevant erythropoiesis in the setting of AZT therapy[73]

MALNUTRITION

Vitamins deficient and other micronutrients is often seen in Humanimmunodeficiencyvirus patients. According to Paltiel et al laboratory outcome in HIV infected persons shows vitamin B12 as low level in up to 30% [74]. This may be due to intestinal malabsorption seen most commonly in these patients. In a study by Matthew et al 13% had at least one low B12 level during the course of their infection[75]. In the study by Rule et al serum B12 in 218 asymptomatic HIV positive patients were significantly lower than of a HIV negative control group (P = 0.02). Falling CD4 counts were correlating with low vitamin B12 levels in this study[76]. In the study by Burkes et al done in HIV positive patients low B12 concentration was not correlating with macrocytosis and parenteral vitamin B12 replaced mostly failed to elevate serum cobalamin levels[78]. This may be because of an early B12 deficiency or it may reflect an isolated serum abnormality. While intestinal malabsorption may also be contribute for low cobalamin level, in majority of patients the cause is notknown. This also be due to altered transcobalamin activity and further studies are being done to evaluate low vitamin B12 levels without hematological manifestations[77] Fuchs et al have observed a decreased in folate level of 27% [79].

True serum Fe deficiency is often seen in Humanimmunodeficiencyvirus patients[79] More commonly decreased serum iron is due to functional block in iron release. Bone marrow examination has exposed as enough or increased Fe stores thus leading to the Fe usage in HIV diseases may also altered a functional block in iron release.

Altered iron metabolism in HIV

Humans with advanced (HIV) diseases present in some witness says of Fe increased. High level Fe may exert negative effects in persons because of Fe-mediated oxidative stress. Iron-mediated oxidative stress is mostly likely involved in viral cytopathogenicity. This evidence suggests that Fe metabolism is an significant area for virus & host interaction [80]. Large number of patients in more advanced stages of HIV have more iron deposits in the bone marrow, liver, and also other organs by one or more various several mechanisms.These various ways added the sequestration of Fe upon macrophages with chronic inflammation , a change of Fe from Hb in red

cells to various macrophage stores at the time of the development of hypoplastic anemia, the transfusion of blood , the increase of Fe acquisition related with ART drugs causing not effective erythropoiesis and a other types inherited or acquired iron-loading conditions. [81, 82]

HEMOLYTIC ANEMIA

In 37% of HIV-infected persons are positive with direct coobs test and to have clinically hemolysis is rare [83] This shows that positive DCT in HIV-infection may simply be a result of polyclonal hypergammaglobulinemia that are verycommon in HIV infected people and does not necessarily indicate hemolysis. A most of specific and nonspecific antierythrocyte antibodies also been detected in those patients. Anti-I, anti-U, anti-E and anti-K antibodies are more commonly detected [84] [85]. One study says complement receptor induced destruction of red cells by on flowing immune complexes [86]. With in all HIV patients with autoimmune hemolytic anemia says reduced reticulocyte count quitly interesting[87]

BLOOD LOSS

Gastrointestinal involvement in AIDS may also one of the etiology for anemia due to blood loss . Infections like CMV colitis & malignancies like Kaposi's sarcoma related to significant anemia and also due to GI blood wastage. In developing countries risk of anemia are may vary from the developed countries in various ways like endemic undernutrition, worm infections, TB and a various spectrum of opportunistic diseases. In the study by Subbaraman et al female sex, extrapulmonary & pulmonary TB very highly independent interlink with anemia. Age more than tnirteen one years, oral,oesophageal candidiasis & generalized lymphadenopathy had independent risk factor with anaemia[3] Three of various influencial things associated with anemia – TB, immunosuppression and undernutrition – shows each other in synergistic manner. The final result is vicious cycle seen in HIV positive patients in TB-endemic countries at very high risk anemia to develop.

TREATMENT

The diagnostic approach to anemia in Humanimmunodeficiencyvirus infected subjects were also be performed with other causative conditions. However the treatment of anemia in HIV positive patients to differ from HIV negative persons as the etiologies may differ especially in patients with severe immunodeficiency. The clinical workup say HIV diseased patients with anemia must be include an attempt to rule out all correctable causes of anemia . After correction of the underlying cause(s) the management of anemia typically includes and blood transfusion or erythropoietin.

Treatable causes of anemia in HIV positive patients[89]

- Nutritional deficiencies(malnutrition/malabsorption)
- Anemia of chronic disease
- Myelosuppresive drugs
- Vitamin B12,iron or folate deficiency
- Neoplasia(eg Non Hodgkins lymphoma)
- Oppurtunistic bone marrow infections

HAART.

HAART gives result to very well improvement of various anemia. A different results from the WIHS study says that HAART found crucially related with anemia to corect; outcome was noted around six months, and a major resolution occurred long time intake of a HAART use (P <.0001) [90.] In the setting of a generalized HIV epidemic HIV-complicated anemia requires a various approach. HAART can be initiated for the anemia if no reason is identified for cause [95]. The 2010 revision of the WHO guidelines for resource-limited strongly recommend to start ART with a particular regimen wih zidovudine or tenofovir in joining with lamivudine & a non-nucleoside reverse transcriptase inhibitor, because of toxic effect due to long-term toxicities of stavudine[97]

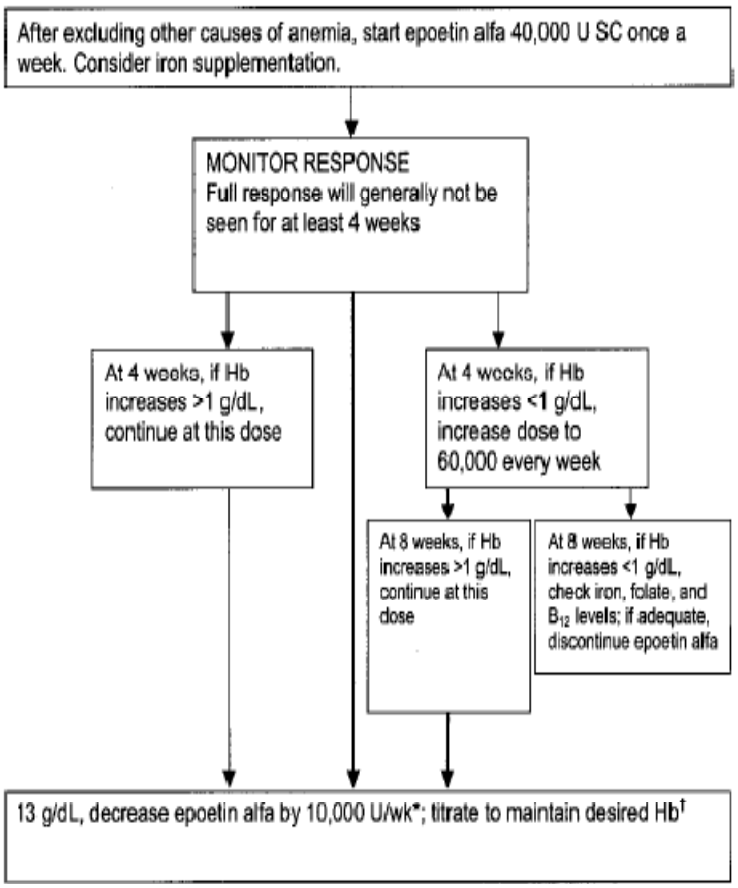
BLOOD TRANSFUSION

Therapies like anti-anemic increase the effect of HIV disease. Blood transfusions also reasons to have also useful as immunomodulatory effect by up regulation of humoral immunity and downregulating cellular immunity. Blood transfusions may also happen to decline in T helper type1 & increased T helper type 2 cytokine production as well as increased CD 8 cells and decreasd CD4 cells[91]

ERYTHROPOIETIC AGENTS

In multiple controlled and uncontrolled study shows results, epoetin alfa action shows effective and important role for the anemia treatment in HIV infection. Epoetin alfa marked improved hematocrit values (P=0.05) in persons with HIV and are all taking zidovudine, with endogenous erythropoietin levels of <500 IU/ L. An incline 11 g was seen by a week [2], furthers increase the level of 12 g by week [4]. Treatment found to be related with marked reductions in blood transfusion requirements (P<0.003) and aberrantly increase the overall quality of life [92] [93]. Increased with levels of proinflammatory cytokines, EPO show little set back in poor response in anemia ,one side and decrease Fe availability on other side . In clinical terms, corrected reticulocyte counts are used more to know the response of EPO level and also potential response and not responding. However in comparison with placebo human recombinant EPO no change in mortality, no change in blood transfusion requirements, not increase Hb values and same not good outcome with quality of life for HIV positive persons with anemia according to the Cochrane review done based on six trials [96.] Therefore prescription for human EPO for anemia to treat in HIV patients is not approved unless, high quality trial is not alters this results with no other new evidence results found .

FIGURE 11: GUIDELINES FOR ERYTHROPOIETIN IN HIV POSITIVE PATIENTS[94]



In addition to role of HAART, other supportive & TB control should be the good work for anemia management for HIV individuals in India[88]. Strongest factors are three thing with anemia – TB, immunosuppression and undernutrition – all exacerbate one other in same manner. The overall result is a vicious cycle placing HIV- patients in Tuberculosis-endemic countries at more risk for complication of anemia.. Low Body Mass Index is are also related with many micronutrient deficiencies – including Fe, folate and B12 – that contribute directly to anemia.

FUTURE TRENDS

Pro inflammatory cytokines TNF alpha and IL beta possibly happens to decreased renal erythropoietin synthesis. Interventional studies with substances that have stop release of proinflammatory cytokines such as pentoxyphylline have no response to treat the anemia inducing effects.[98] Future work up should focus on to understand various reasons of anemia, long-term result to give results and prognostic values, the results of various HAART regimens the overall case load of anemia, and valuable dosing are strategies to treat with epoetin alfa in all type of special group of people. Emerging data shows results as that epoetin alfa has valid effects more than erythropoiesis. There is also evidence, for evidence, that epoetin alfa also has antiapoptotic effects in various multiple cell lines,and also helpful in way of all have a good impact on the immune response in individuals with HIV infection .

4. METHODOLOGY

SETTING

This was a descriptive cross-sectional study done during the period of April 2016 to September 2016 on 60 consecutive HIV patients in different stages of disease seen as inpatients in medical ward MADRAS MEDICAL COLLEGE (MMC), CHENNAI, TAMIL NADU, INDIA.

All respondents were adults, aged more than 18 yrs. Informed consent was taken prior to inclusion in the study.

PATIENTS

Patients were selected on an in-patient basis in medical ward. The in-patients were those who were admitted to the medical wards of MMC during the aforementioned period

INCLUSION CRITERIA

HIV positive patients

Anemia with hemoglobin less than 10.9gm/dl

METHOD OF COLLECTION OF DATA

All patients were interviewed and examined by the investigator. A Proforma, which is appended in the annexure, was used for the above purpose.

TESTS

HIV was confirmed by the ELISA test. CD4 counts were analyzed using the Flowcytometry method. Hemoglobin, total count and differential count were performed in the laboratory using automated counting chambers and confirmed manually. Further work up for anemia including reticulocyte count, peripheral smear examination, mean corpuscular volume estimation, direct coombs test, serum ferritin and B12 levels were done. Bone marrow aspiration was done for most of the patients as part of anemia evaluation. Other tests were done as per the needs of the patient.

STATISTICAL METHODS

Statistical analyses was performed with SPSS. Normal data were summarized using mean and standard deviation (SD) and non-normal data using median and interquartile range. Correlation coefficient was be used to compare hemoglobin values and CD4 counts.

5. RESULTS

DEMOGRAPHY

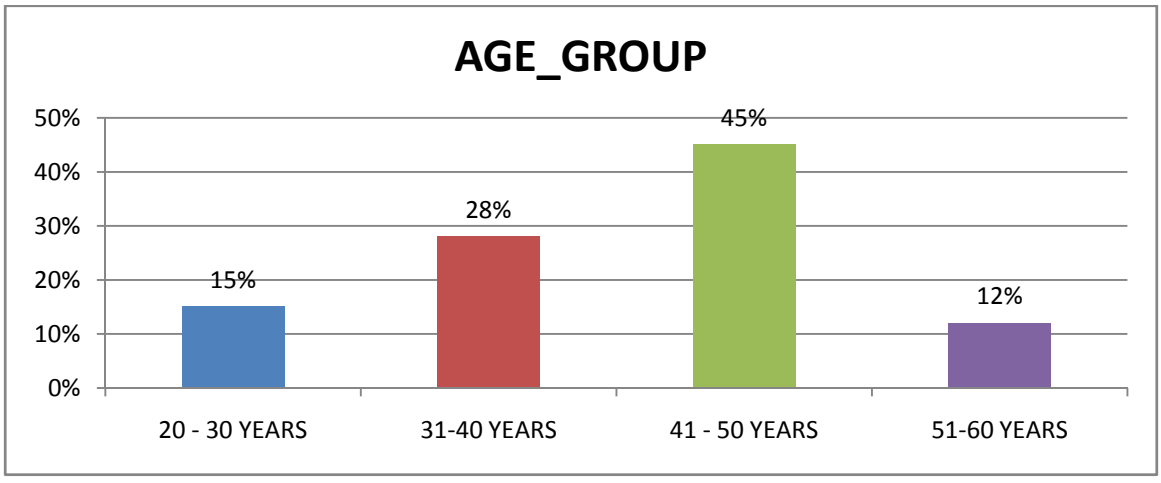
Table:2 AGE DISTRIBUTION

AGE_GROUP	FREQUENCY	PERCENT
20 - 30 YEARS	9	15.0
31-40 YEARS	17	28.3
41 - 50 YEARS	27	45.0
51-60 YEARS	7	11.7
Total	60	100.0

Table : 3 AGE DISTRIBUTION

Descriptive Statistics	N	Minimum	Maximum	Mean	Std. Deviation
AGE	60	22.00	60.00	41.2333	8.52785

Chart : 1 AGE DISTRIBUTION

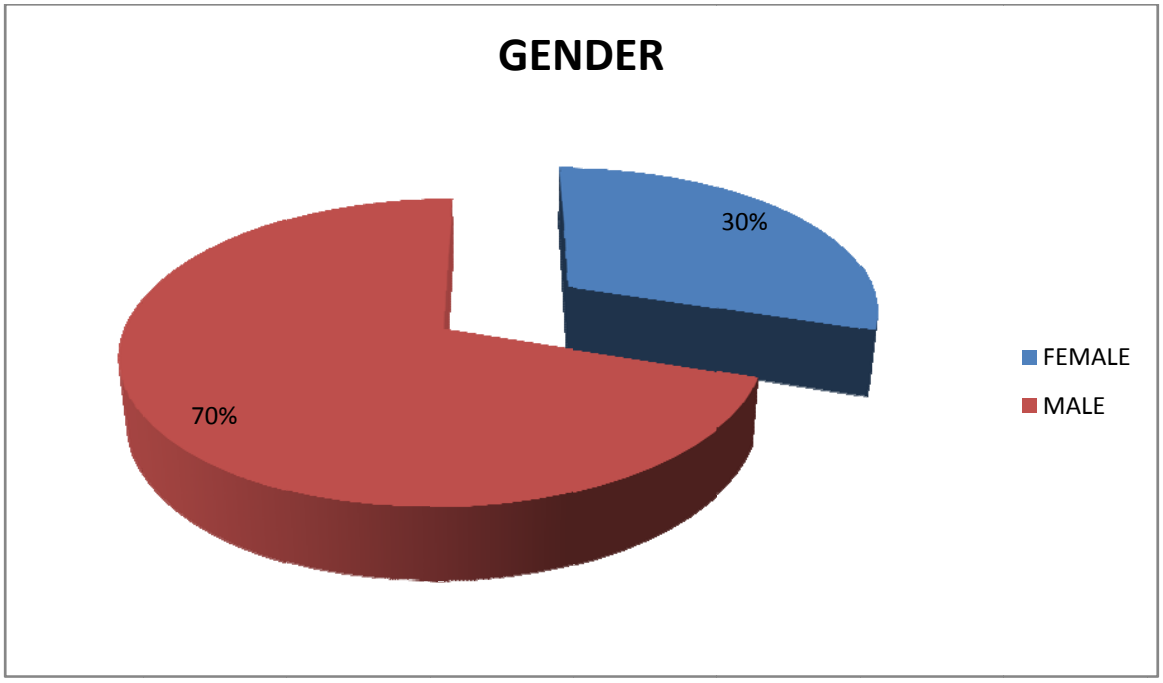


SIXTY PATIENTS WITH HIV INFECTION AND ANEMIA WERE INCLUDED IN THE STUDY.MINIMUM AGE WAS 22 YEARS AND MAXIMUM AGE WAS 60 YEARS (MEAN±SD: 41.23±8.52)

Table :4 GENDER DISTRIBUTION

SEX	FREQUENCY	PERCENT
FEMALE	18	30.0
MALE	42	70.0
Total	60	100.0

Chart :2

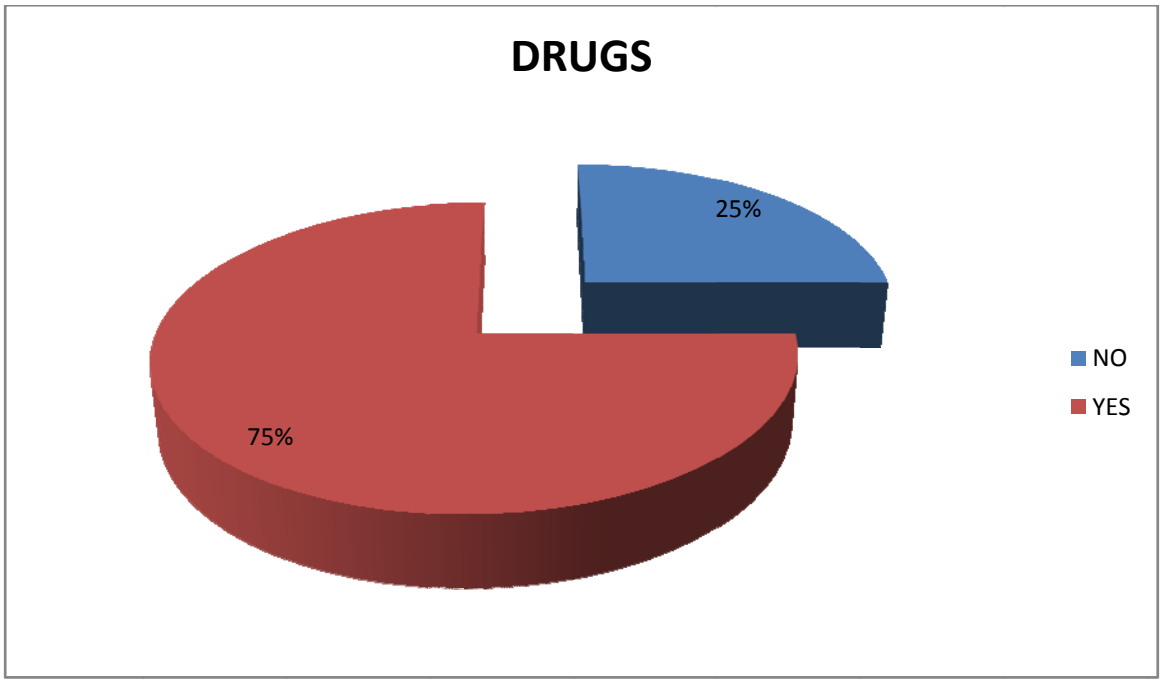


70% (42) OF THE SUBJECTS WERE MALE AND 30% (18) WERE FEMALE

Table: 5 FREQUENCY OF PATIENT ON CO TRIMOXAZOLE

DRUGS	FREQUENCY	PERCENT
NO	15	25.0
YES	45	75.0
Total	60	100.0

Chart :3

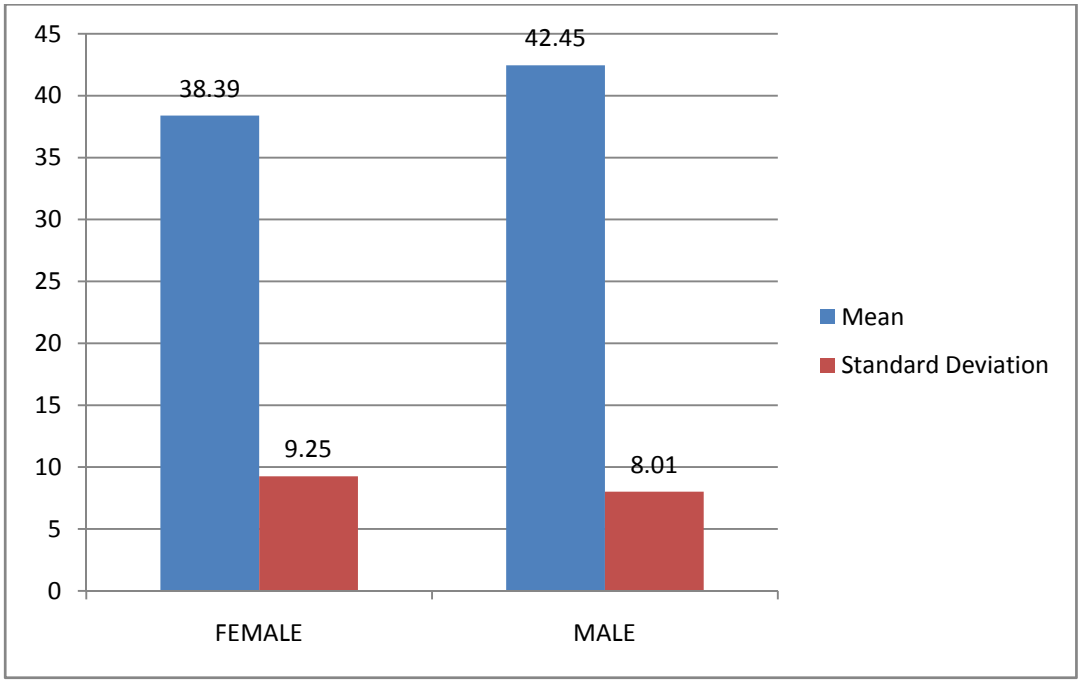


75% OF PATIENT ON CO-TRIMOXAZOLE AND 25% NOT ON CO-TRIMOXAZOLE

Table :6 MEAN IN AGE

AGE	MEAN	STANDARD DEVIATION
FEMALE	38.39	9.25
MALE	42.45	8.01
Total	60	100.0

Chart :4

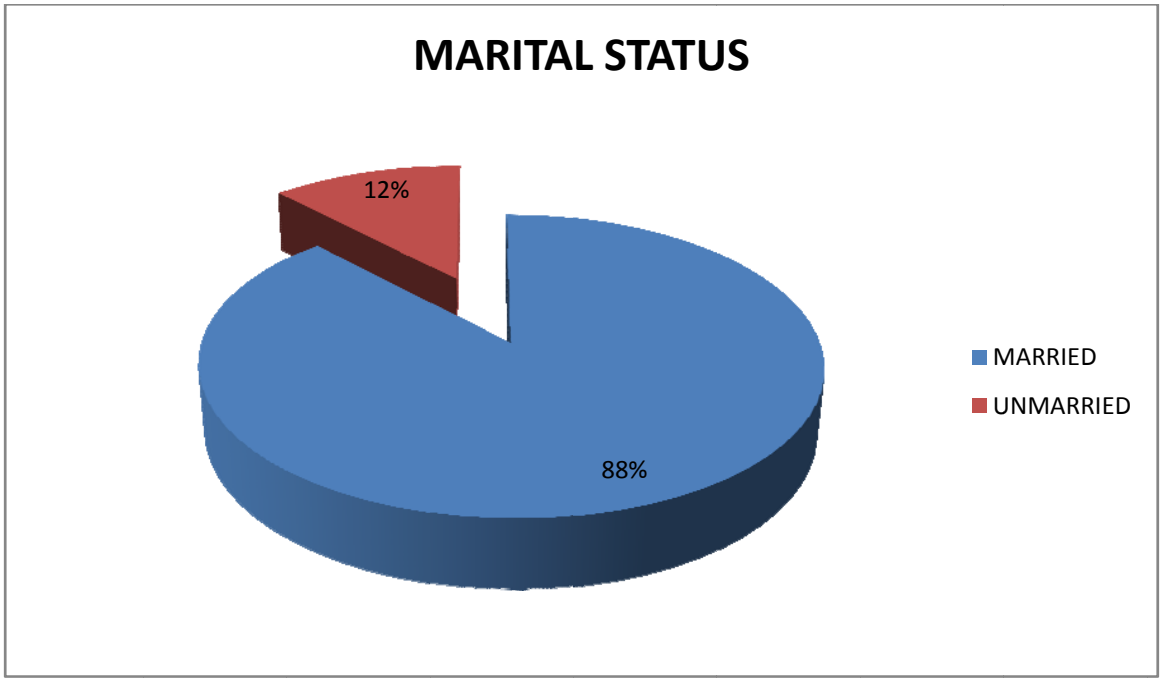


MEAN IN AGE ± SD FEMALE (38.39 ±9.25) MALE (42.45± 8.01)

Table :7 MARITAL STATUS OF PATIENT

MARITAL STATUS	FREQUENCY	PERCENT
MARRIED	53	88.3
UNMARRIED	7	11.7
Total	60	100.0

Chart :5



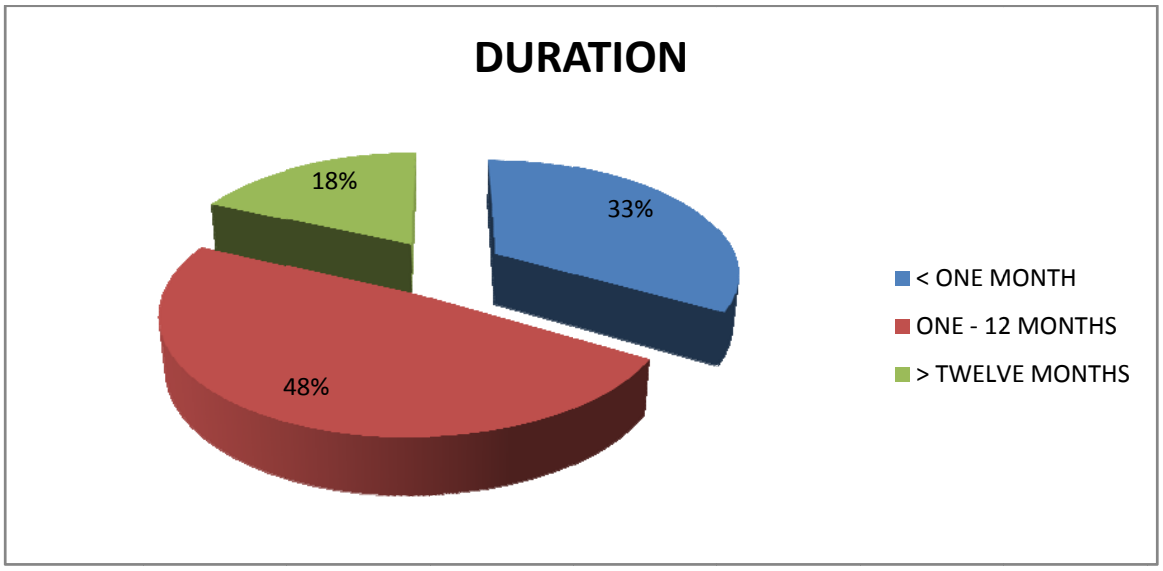
IN THE STUDY 88.3% ARE MARRIED AND 11.7% ARE UNMARRIED

DISEASES CHARACTERISTICS

Table :8 DURATION SINCE DIAGNOSIS

DURATION	FREQUENCY	PERCENT
< ONE MONTH	20	33.3
ONE - 12 MONTHS	29	48
> TWELVE MONTHS	11	18.7
Total	60	100.0

Chart :6



	N	Mean	Std. Deviation	Std. Error
NEWELY DIAGNOSED	20	8.6450	1.56893	.35082
1- 12 MONTHS	29	7.8828	1.24099	.23045
ABOVE 12 MONTHS	11	7.2455	1.08200	.32624
Total	60	8.0200	1.40553	.18145

F = 4.196 * P=0.02

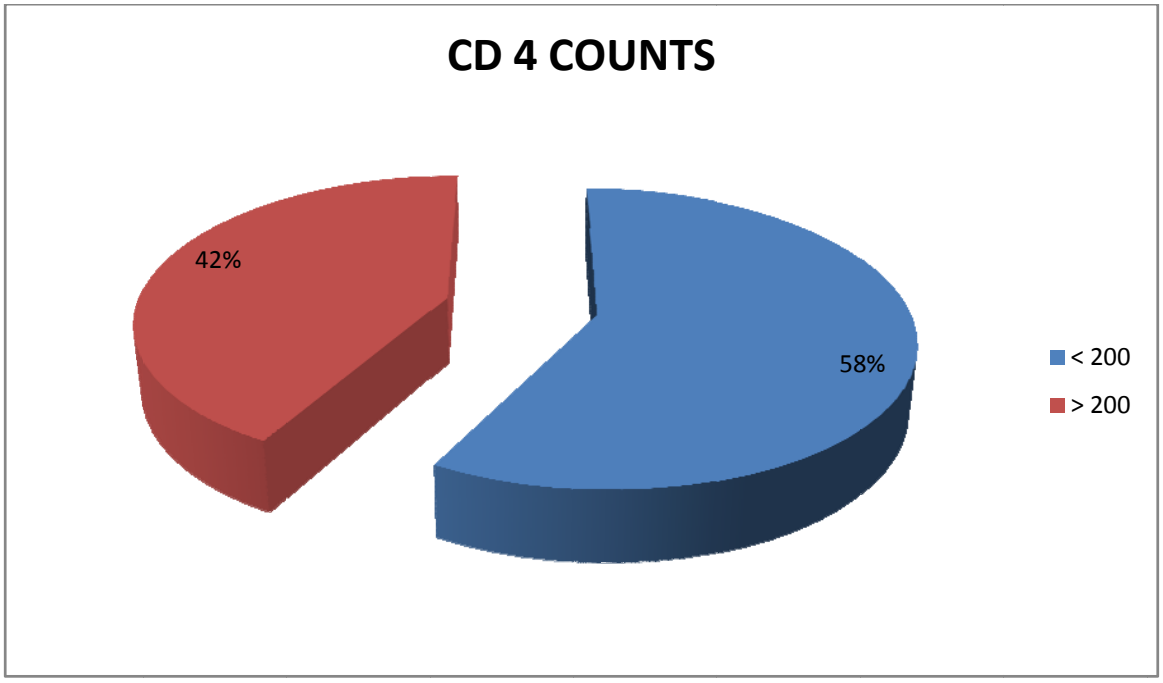
82.3% PATIENTS ARE BELOW ONE YEAR AND 18.7% ARE MORE THAN ONE YEAR

CD4 COUNTS

Table : 9 DISTRIBUTION OF CD4 COUNTS

CD4 COUNTS	FREQUENCY	PERCENT
< 200	35	58.3
> 200	25	41.7
Total	60	100.0

Chart :7



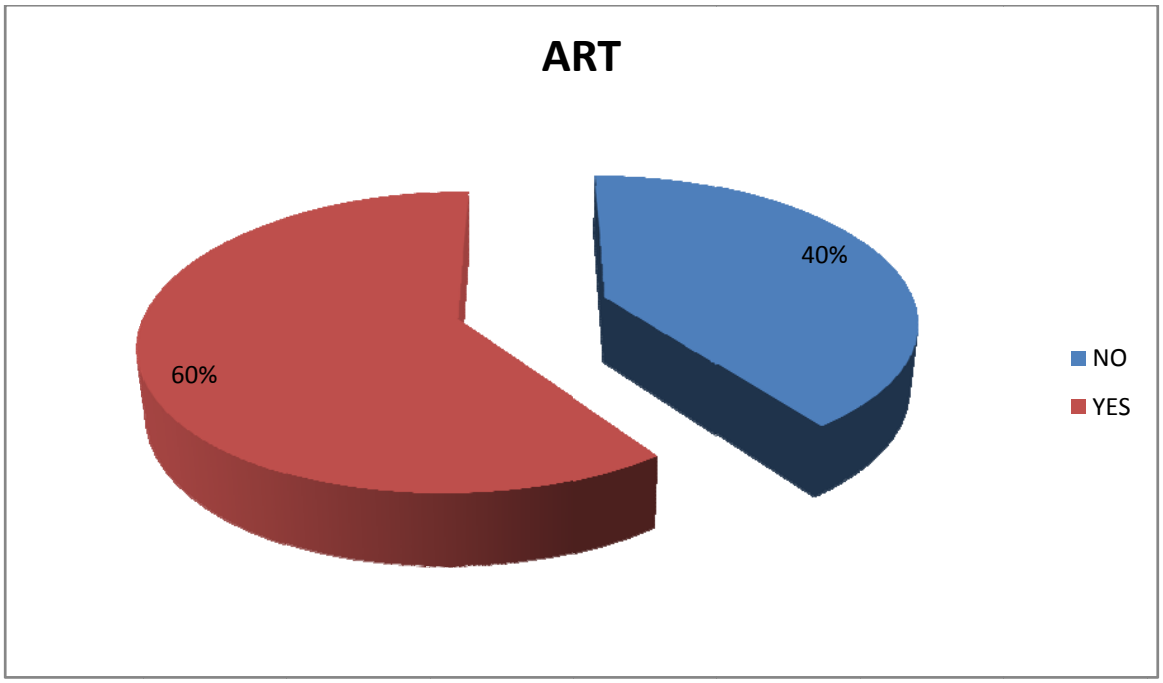
58.3% PATIENTS HAVE <200 AND 41.7% PATIENTS >200,MEAN CD4 COUNT VALUE AROUND 194

THERAPHY FOR HIV INFECTION AND OPPORTUNISTIC INFECTIONS

Table : 10 FREQUENCY OF PATIENTS ON ART

ART	FREQUENCY	PERCENT
N	24	40.0
Y	36	60.0
Total	60	100.0

Chart :8

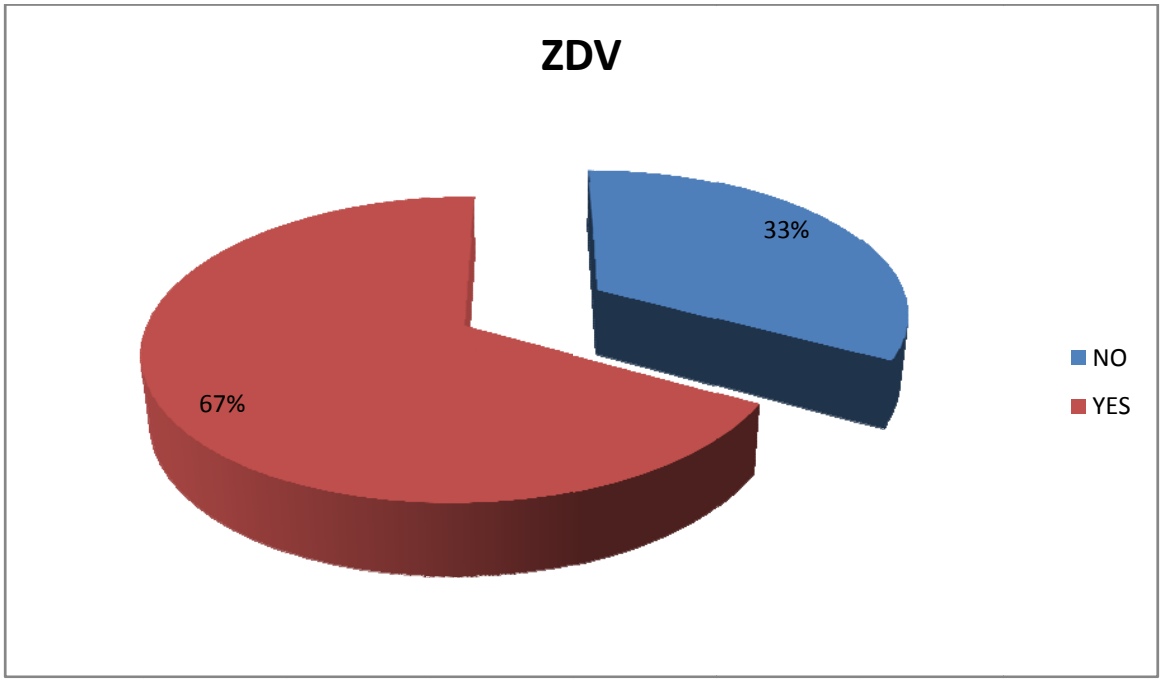


60% OF PATIENTS ON ANTI RETRO VIRAL DRUG (ART)

Table :11 FREQUENCY OF PATIENTS ON ZDV

ZDV	FREQUENCY	PERCENT
N	12	33%
Y	24	67%
Total	36	100.0

Chart : 9

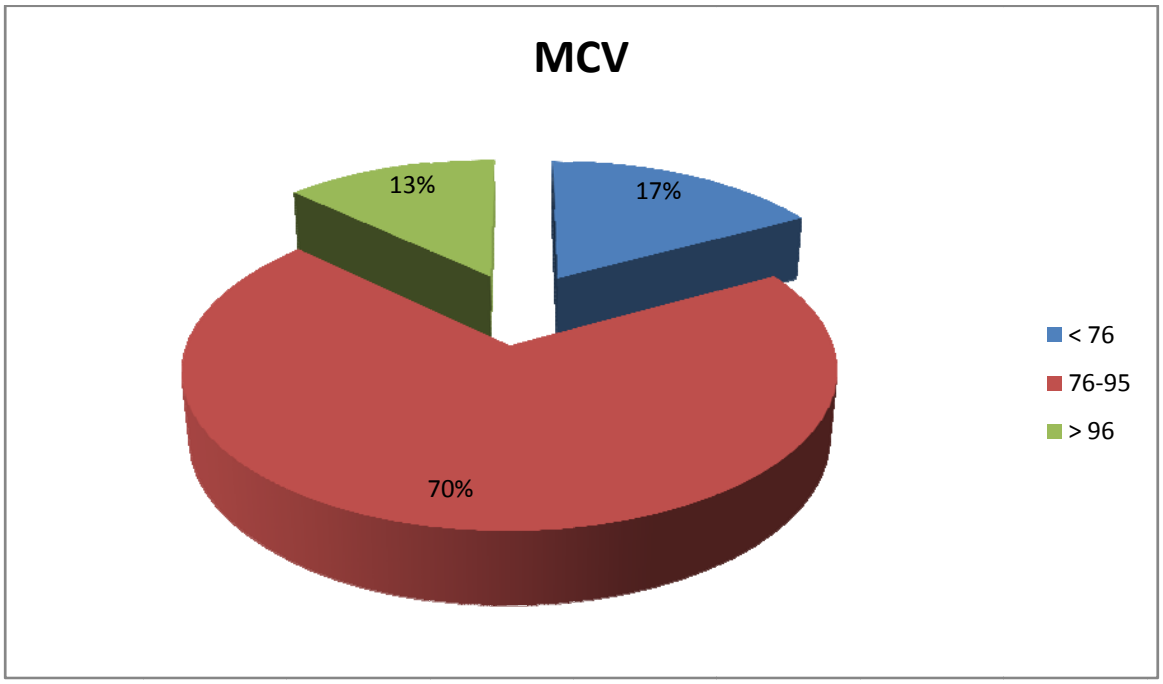


AMONG THE PATIENTS ON ART 67%WAS ON ZIDOVUDINE AND THE REMAINING 33% ON STAVUDINE.THERE WERE NO PATIENT ON ANY OTHER NRTI

Table : 12 MCV LEVELS

MCV	FREQUENCY	PERCENT
< 76	10	16.7
76-95	42	70
> 96	8	13.3
Total	60	100.0

Chart : 10

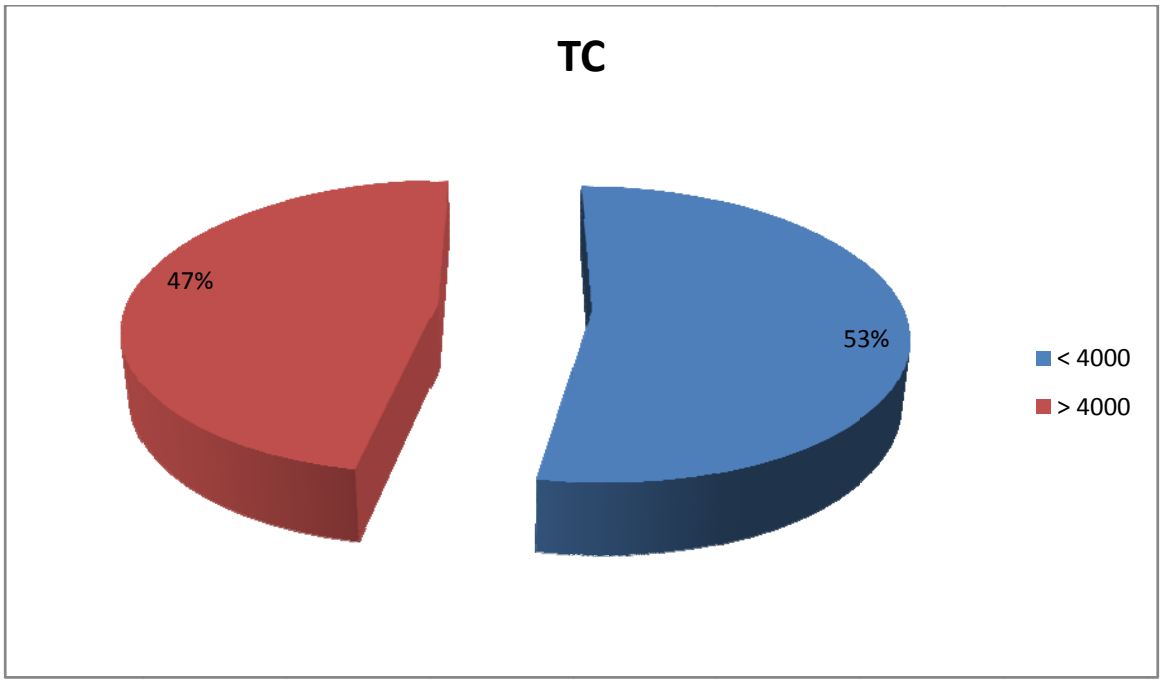


THE MEAN CORPUSCULAR VOLUME WAS 89.2fl(range59-125fl)70% had their MCV IN NORMAL RANGE,16.7 LESS THAN NORMAL,13.3 HAD MORE THAN NORMAL.ONLY 50% PATIENTS WITH VITAMIN B12 DEFICIENCY HAD MACROCYTES.IN PERIPHERAL SMEAR 70% PATIENTS HAD NORMOCYTIC NORMOCHROMIC ANEMIA,22% HAD MICROCYTIC ANEMIA, ANISOPOIKILOCYTES WAS NOTED IN 60% AND TEAR DROP IN 40% OF PATIENTS.

Table : 13 LEUKOPENIA IN HIV PATIENTS

TC	FREQUENCY	PERCENT
< 4000	32	53.3
> 4000	28	46.7
Total	60	100.0

Chart : 11

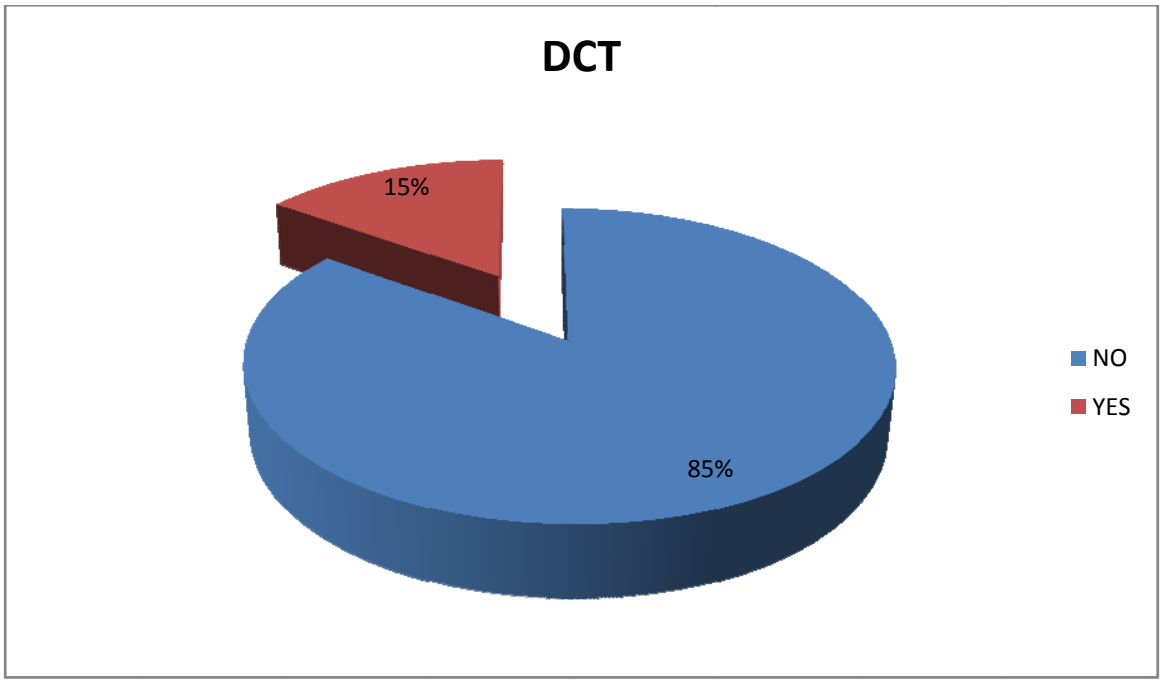


53.3% HAD LEUKOPENIA AND 46.7% HAD NORMAL LEUKOCYTES

Table : 14 DIRECT COOMBS TEST

DCT	FREQUENCY	PERCENT
N	51	85.0
P	9	15.0
Total	60	100.0

Chart : 12



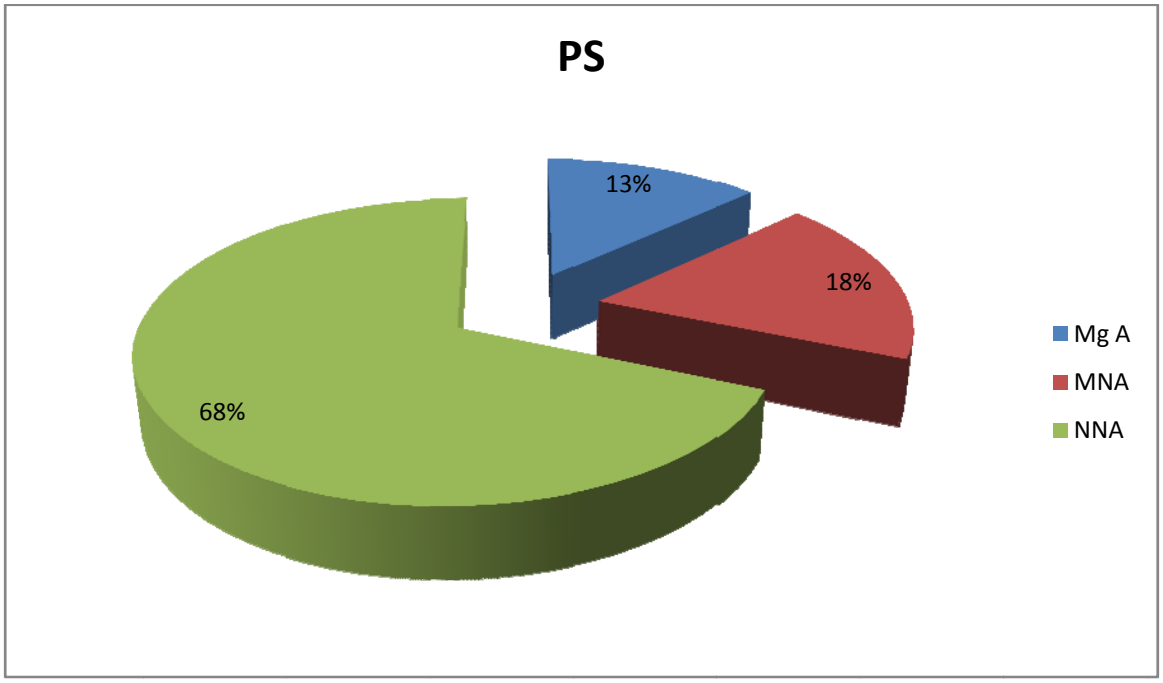
15% OF THE PATIENT HAD POSITIVE DIRECT COOBS TEST.ONLY 3% HAD EVIDENCED OF HEMOLYSIS (HIGH LDH LEVELS, UNCONJUGATED HYPER BILIRUBINEMIA)

Table : 15 PERI PHERAL SMEAR EVALUATION

PS	FREQUENCY	PERCENT
Mg A	8	13.3
MNA	11	18.3
NNA	41	68.3
Total	60	100.0

MgA-MEGALOBLASTIC, MNA-MICROCYTIC, NNA-NORMOCYTIC ANEMIA

Chart: 13



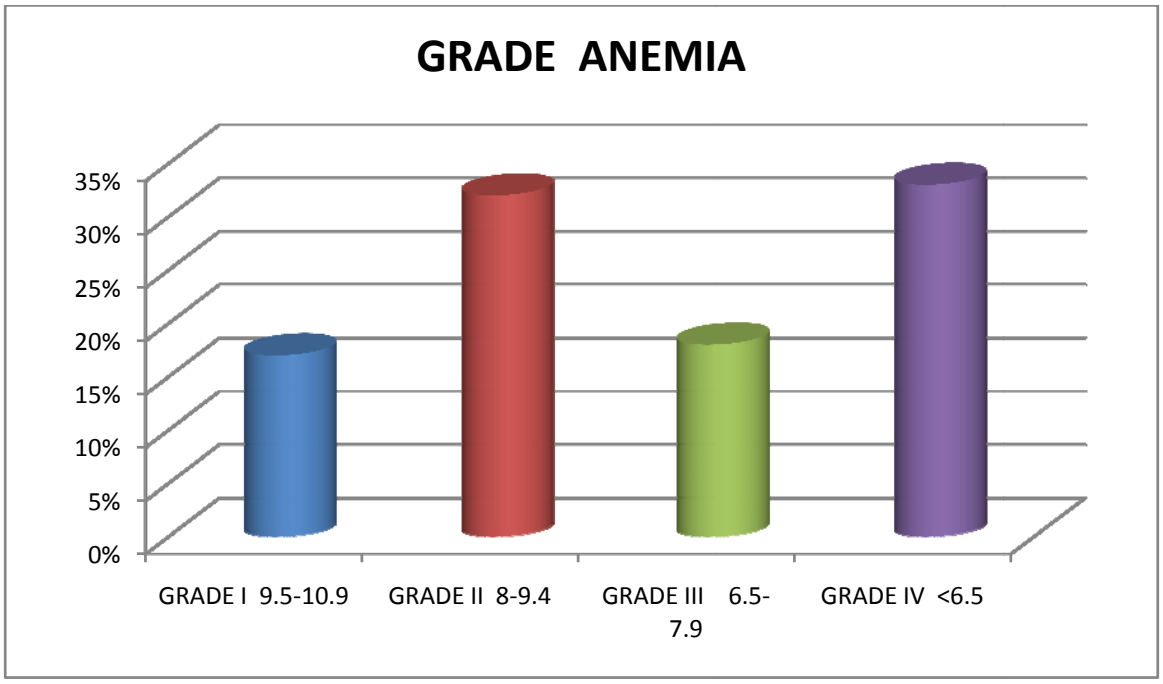
13.3% HAD MEGALOBLASTIC PICTURE, 18.3 % HAD MICROCYTIC AND 68.3% HAD NORMOCYTIC PICTURE

EVALUTION OF ANEMIA

Table : 16 GRADES OF ANEMIA

GRADE OF ANEMIA	FREQUENCY	PERCENT
GRADE I 9.5-10.9	10	16.7
GRADE II 8-9.4	19	31.7
GRADE III 6.5- 7.9	11	18.3
GRADE IV <6.5	20	33.3
Total	60	100.0

Chart : 14

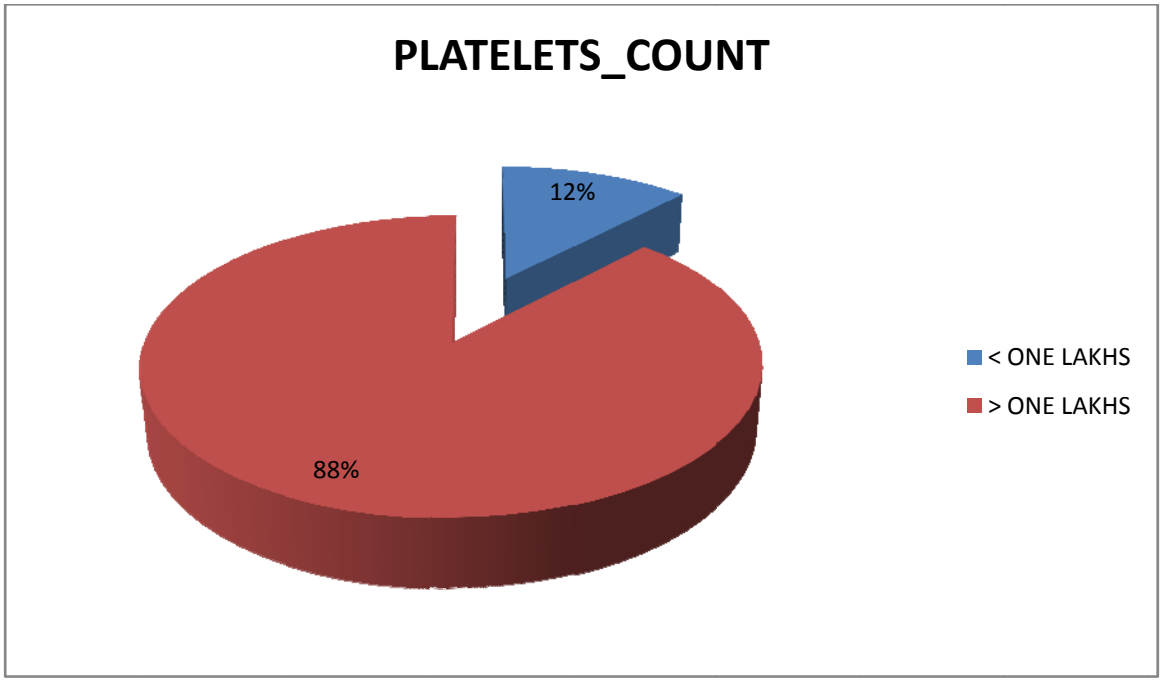


16.7% HAD GRADE ONE, 31.7% HAD GRADE TWO, 18.3% HAD GRADE THREE, 33.3% HAD GRADE FOUR

Table : 17 THROMBOCYTOPENIA IN HIV PATIENTS

PLATELETS COUNT	FREQUENCY	PERCENT
< ONE LAKHS	7	11.7
> ONE LAKHS	53	88.3
Total	60	100.0

Chart :15

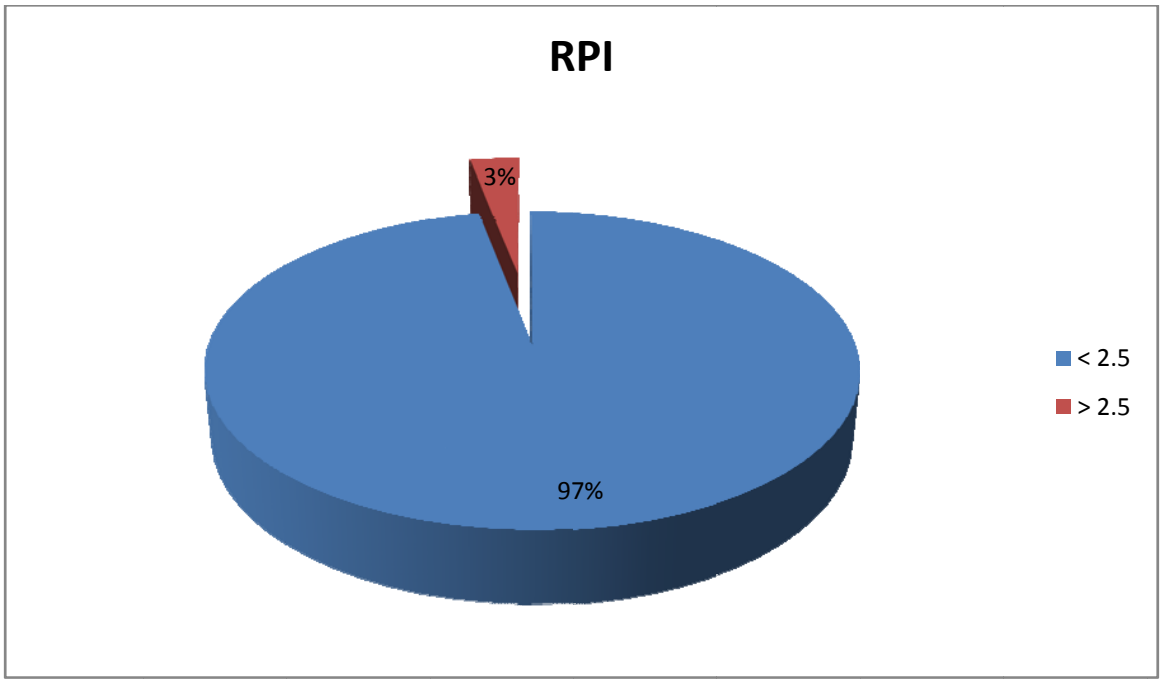


11.7% HAD < ONE LAKH AND 88.3% HAD > ONE LAKHS

Table : 18 RETICULOCYTE PROLIFERATION INDEX

RPI	FREQUENCY	PERCENT
< 2.5	58	96.7
> 2.5	2	3.3
Total	60	100.0

Chart : 16

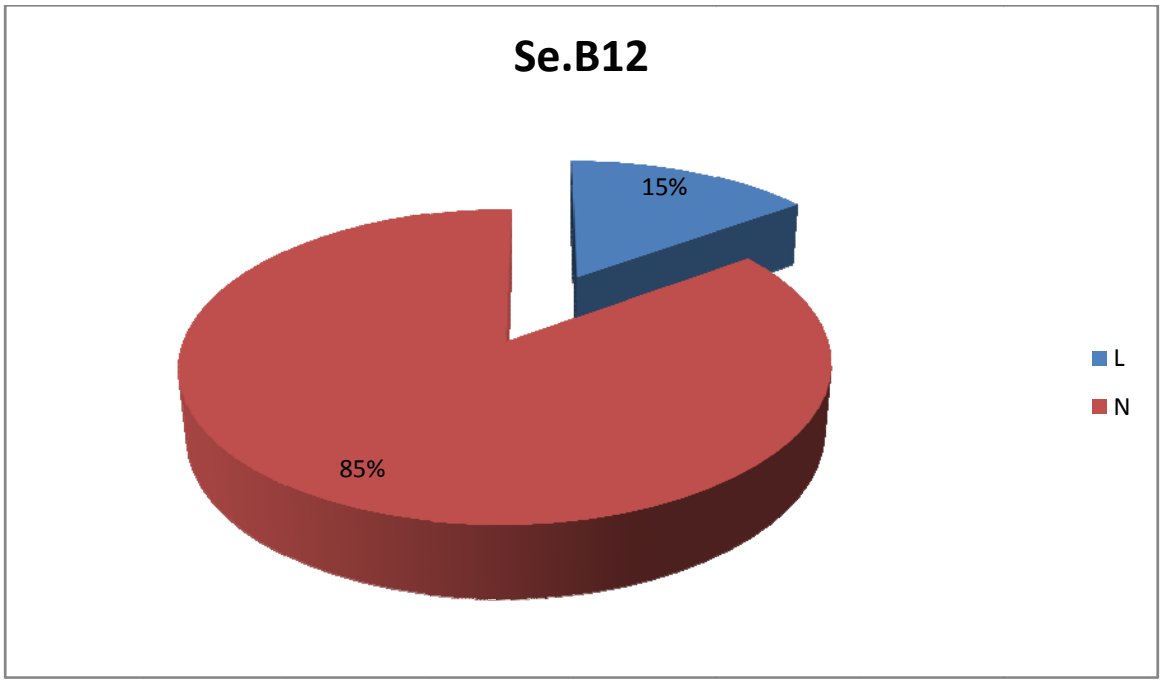


96.7% ASSOCIATED WITH < 2.5 AND 3.3% ASSOCIATED WITH > 2.5

Table : 19 VITAMIN B12 LEVEL

Se.B12	FREQUENCY	PERCENT
L	9	15.0
N	51	85.0
Total	60	100.0

Chart : 17

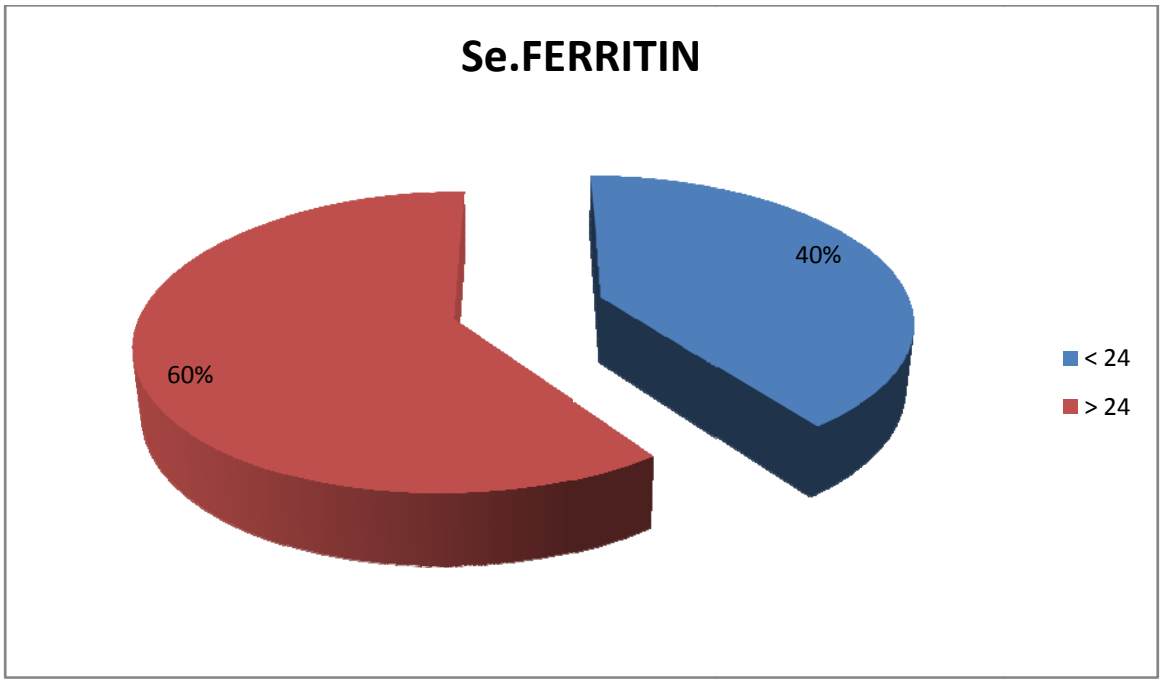


85% HAVE NORMAL LEVEL AND 15% HAD LOW LEVEL, THERE IS NO CORRELATION BETWEEN B12 AND CD4 COUNT AND HEMOGLOBIN

Table : 20 SERUM FERRITIN LEVEL

Se.FERRITIN	FREQUENCY	PERCENT
< 24	2	3.3
> 24	58	96.7
Total	60	100.0

Chart :18



3.3% HAD LOW SERUM FERRITIN LEVELS in our study no correlation between S.ferritin and CD4 count and haemoglobin.though10% of patients our study had iron deficiency anemia, only 3.3% had low ferritin levels

Crosstab

Table : 21 CORRELATION BETWEEN CD4 COUNT AND Se.B12

CD4 COUNT AND B12 LEVEL			CD4COUNTS		Total
			<200	>200	
S.B12	L	Count	7	2	9
		% within CD4COUNTS	20.0%	8.0%	15.0%
	N	Count	28	23	51
		% within CD4COUNTS	80.0%	92.0%	85.0%
Total		Count	35	25	60
		% within CD4COUNTS	100.0%	100.0%	100.0%

Pearson Chi-Square t = 1.647 p= 0.199

Table : 22 Crosstab

CD4 COUNT AND S.FERRITIN			CD4COUNTS		Total
			<200	>200	
S.FERRITIN	< 24	Count	2	0	2
		% within CD4COUNTS	5.7%	0.0%	3.3%
	> 24	Count	33	25	58
		% within CD4COUNTS	94.3%	100.0%	96.7%
Total		Count	35	25	60
		% within CD4COUNTS	100.0%	100.0%	100.0%

Pearson Chi-Square t =1.478 p=0.224

Group Statistics

Table : 23 CORELATION BETWEEN Hb and ART

	ART	N	Mean	Std. Deviation	Std. Error Mean
Hb	NO	24	8.4625	1.51910	.31008
	YES	36	7.7250	1.26092	.21015

t=2.044* p=0.045

Chart : 19

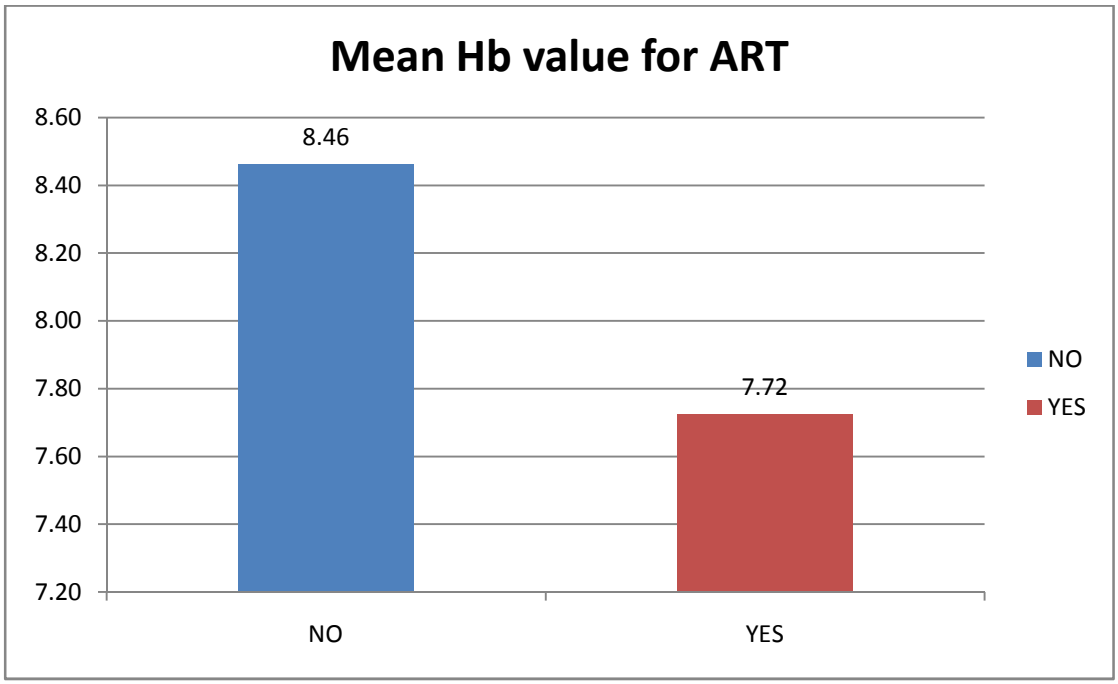


Table : 24 CORRELATION BETWEEN Hb and ZDV

ZDV	FREQUENCY	PERCENT
N	16	45%
Y	20	55%
Total	36	100.0

Chart :20

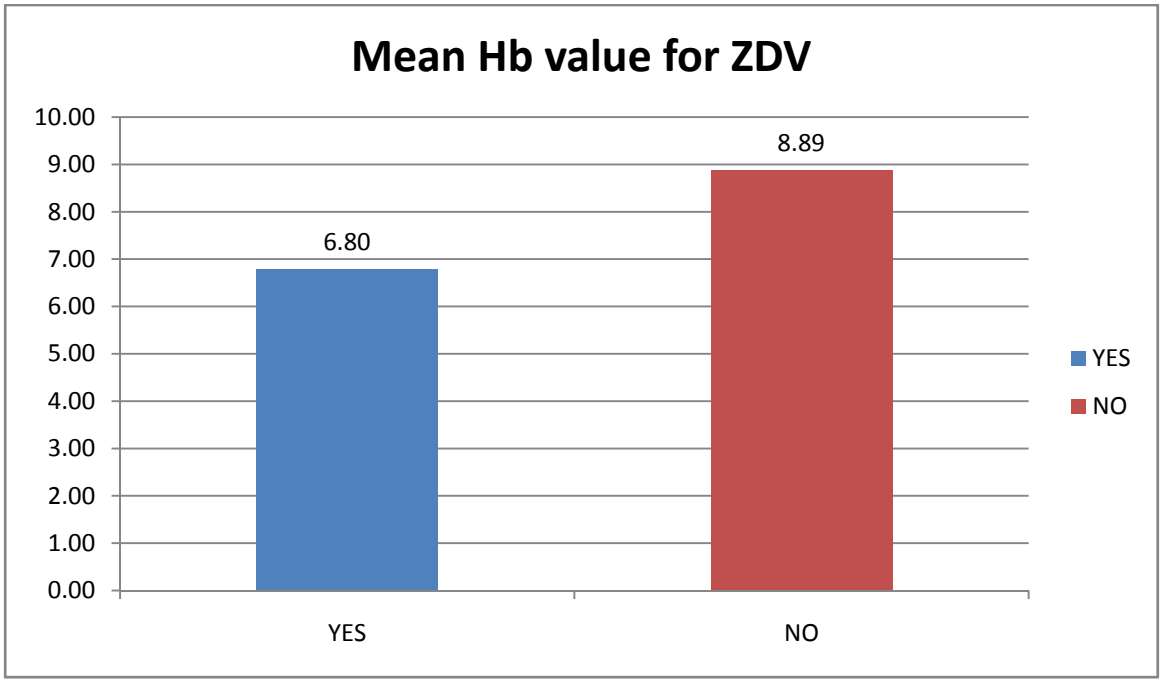


Table : 25 CORRELATION BETWEEN MCV AND ART

	ART	N	Mean	Std. Deviation	Std. Error Mean
MCV	NO	24	85.21	7.656	1.563
	YES	36	94.83	17.958	2.993

t=2.475 * p=0.016

Chart : 21

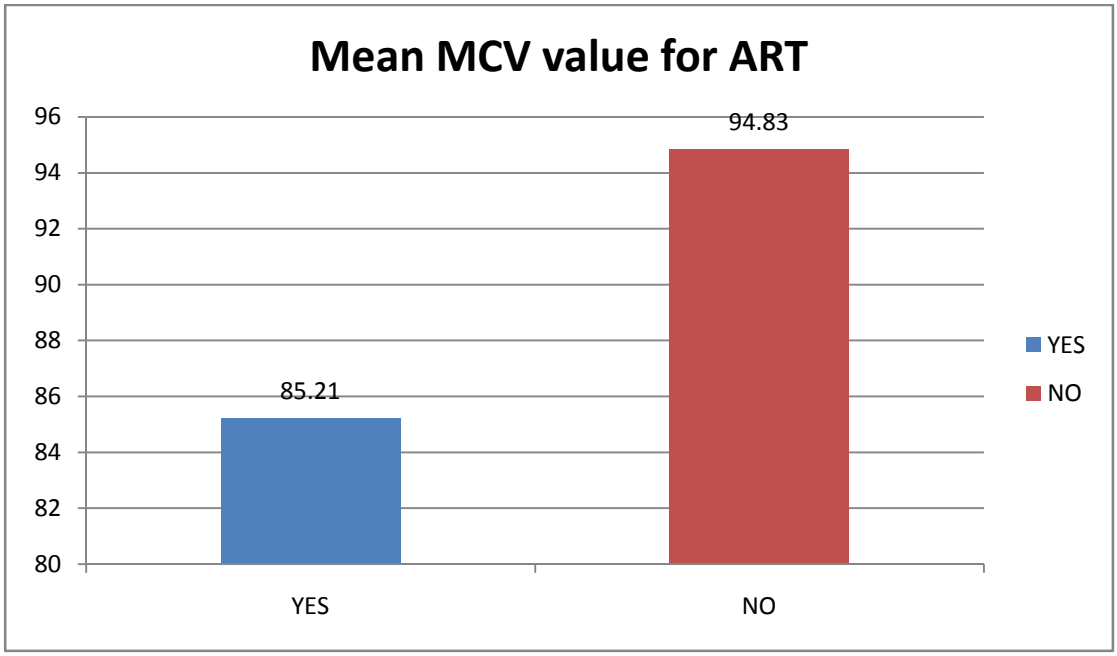


Table : 26 CORRELATION BETWEEN MCV AND ZDV

	ZDV	N	Mean	Std. Deviation	Std. Error Mean
MCV	YES	20	97.55	18.785	4.201
	NO	16	91.44	16.832	4.208

t=1.015 p= 0.317

ANEMIA AND OTHER FACTORS

Table : 27 CORRELATION BETWEEN SEX AND Hb

Group Statistics

	SEX	N	Mean	Std. Deviation	Std. Error Mean
Hb	MALE	42	7.8119	1.41804	.21881
	FEMALE	18	8.5056	1.28451	.30276

t=1.784 p= 0.08

Table 28 Group Statistics ETIOLOGY AND Hb

	ETIOLOGY	N	Mean	Std. Deviation	Std. Error Mean
Hb	OTHERS	29	8.2966	1.51221	.28081
	INFLAMMATION	31	7.7613	1.26798	.22774

t=1.489 p= 0.142

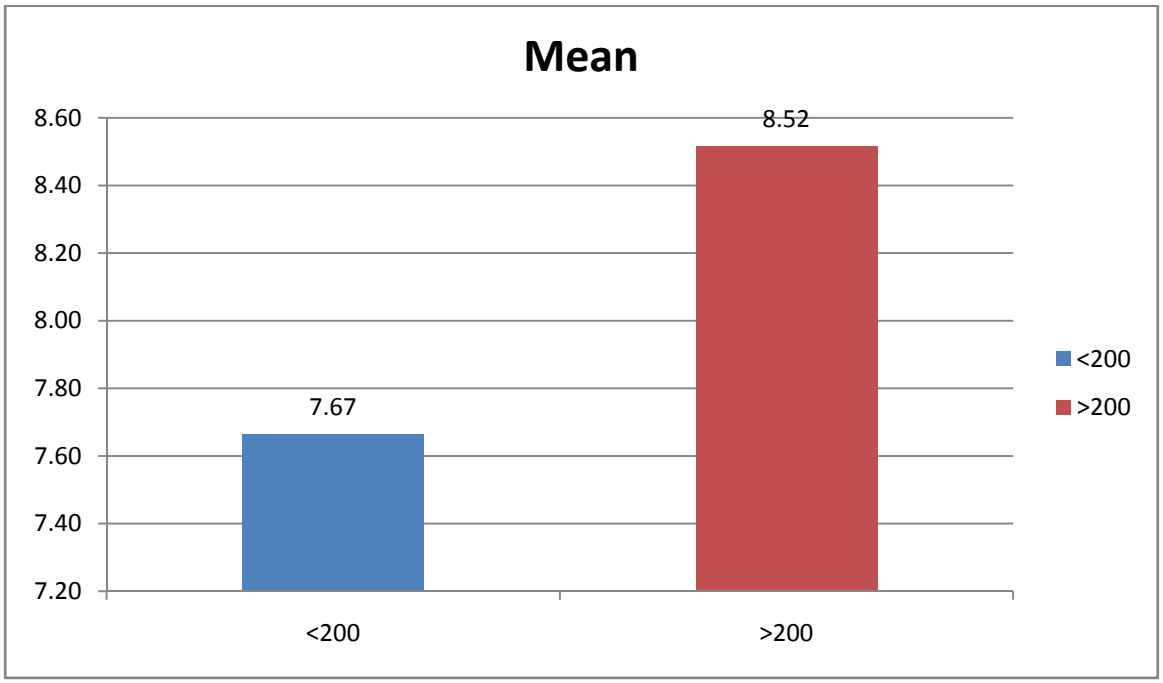
CD4 COUNT AND ANEMIA

Table : 29 CD4 COUNT AND HEMOGLOBIN

	CD4__COUNTS	N	Mean	Std. Deviation	Std. Error Mean
Hb	<200	35	7.6657	1.15248	.19480
	>200	25	8.5160	1.59235	.31847

t=2.042* p=0.02 significant

Chart : 22



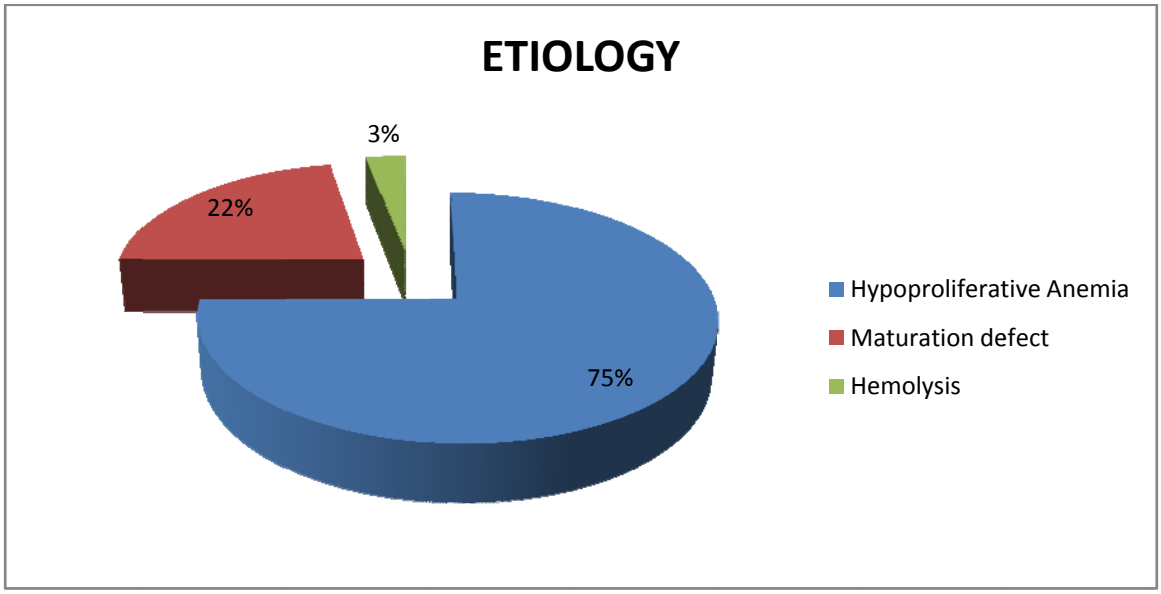
THERE IS SIGNIFICANT DIFFERENCE IN HEMOGLOBIN VALUES AMONG PATIENTS WHO HAD CD4<200 AND CD4 > 200.

ETIOLOGYOF ANEMIA

Table : 30 FUNCTIONAL CLASSIFICATION

FUNCTIONAL ETIOLOGY	FREQUENCY	PERCENT
Hypoproliferative Anemia	45	75.0
Maturation defect	13	21.7
Hemolysis	2	3.3
Total	60	100.0

Chart: 23



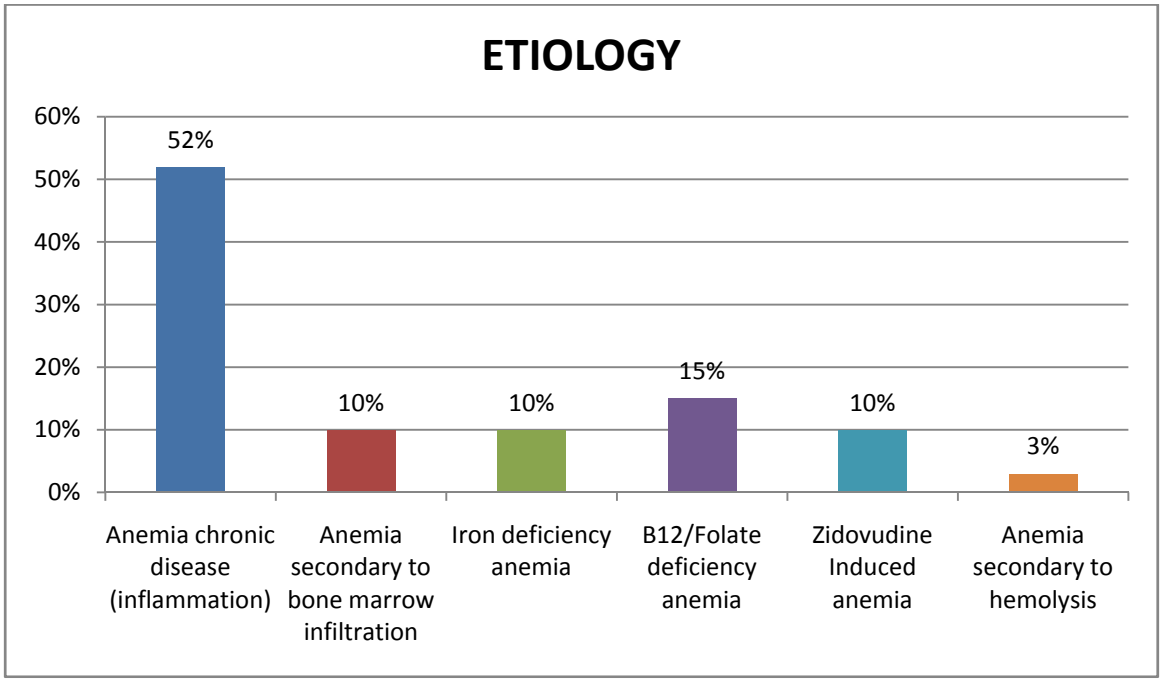
ANEMIA CAUSES	N	Mean	Std. Deviation	Std. Error
Hypoproliferative Anemia	45	7.8000	1.32339	.19728
Maturation defect	13	8.5154	1.48147	.41088
Hemolysis	2	9.7500	1.34350	.95000
Total	60	8.0200	1.40553	.18145

t= 3.076 P=0.054

Table : 31 ETIOLOGY OF ANEMIA

ETIOLOGY	FREQUENCY	PERCENT
Anemia chronic disease (inflammation)	31	51.7
Anemia secondary to bone marrow infiltration	6	10.0
Iron deficiency anemia	6	10.0
B12/Folate deficiency anemia	9	15.0
Zidovudine Induced anemia	6	10.0
Anemia secondary to hemolysis	2	3.3
Total	60	100.0

Chart : 24



Hypoproliferative anemia's is characterized by low reticulocyte production index normocytic, normochromic red cells, although microcytic, hypochromic cells may be observed. Causes are (1) marrow damage, (2) iron deficiency, (3) inadequate EPO stimulation- impaired renal function, suppression of EPO production by inflammatory cytokines such as interleukin 1, or reduced tissue needs for O2 from metabolic disease such as hypothyroidism

Maturation defect is characterized by low reticulocyte production index, macro- or microcytosis on peripheral blood smear. Bone marrow examination shows erythroid hyperplasia. Causes are nuclear maturation defects, associated with macrocytosis and abnormal marrow development or cytoplasmic maturation defects, associated with microcytosis and hypochromia usually from defects in hemoglobin synthesis.

Hemolytic anemia is characterized by increased reticulocyte proliferation index.

CD4 COUNTS AND OTHER PARAMETERS CORRELATION

Table : 32 CD4 COUNT AND ETIOLOGY OF ANEMIA

CD4 COUNT	N	Mean	Std. Deviation	Std. Error	Minimum	Maximum
Hypoproliferative Anemia	45	158.0667	102.63293	15.29961	50.00	608.00
Maturation defect	13	281.7692	202.17078	56.07209	18.00	658.00
Hemolysis	2	270.0000	212.13203	150.00000	120.00	420.00
Total	60	188.6000	140.64033	18.15659	18.00	658.00

t= 4.794*p=0.012

Chart : 25

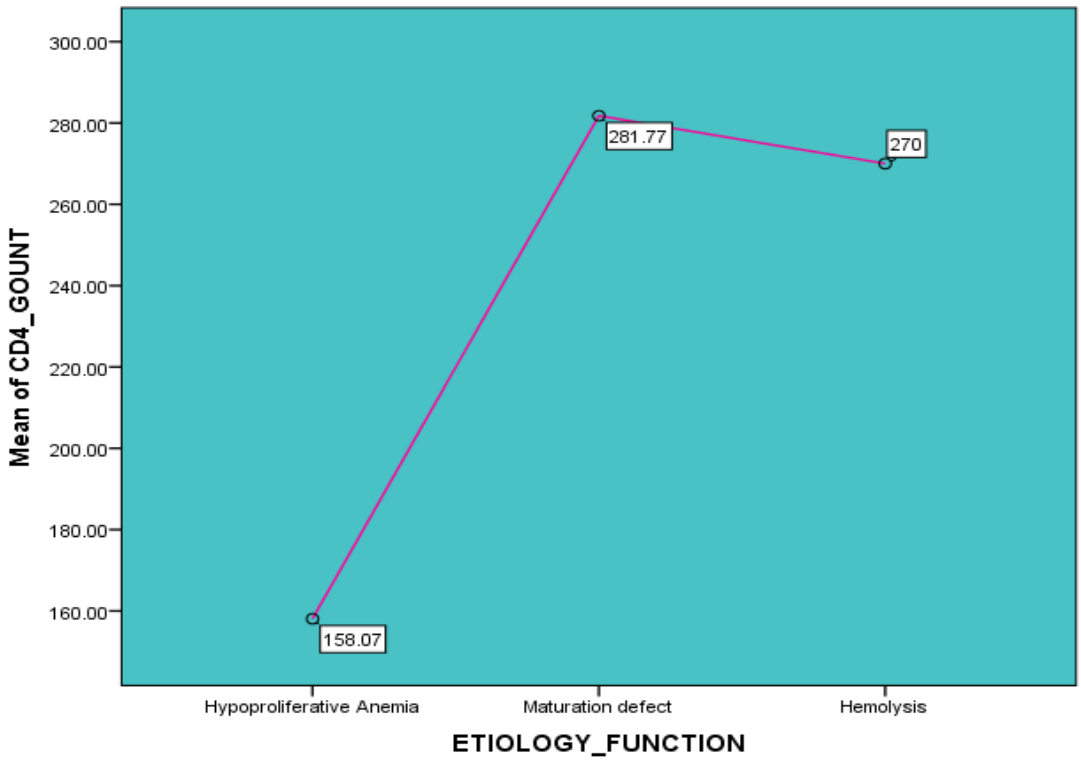


Table : 33 CD4 COUNTS AND VARIES FACTORS OF ANEMIA

GROUP STATISTICS					
	ETIOLOGY	**N**	**Mean**	**Std. Deviation**	**Std. Error Mean**
CD4 COUNT	Anemia chronic disease (inflammation)	31	154.0000	99.86457	17.93621
	OTHERS	29	225.5862	168.08066	31.21180

t= 2.021* p=0.048

Chart : 26

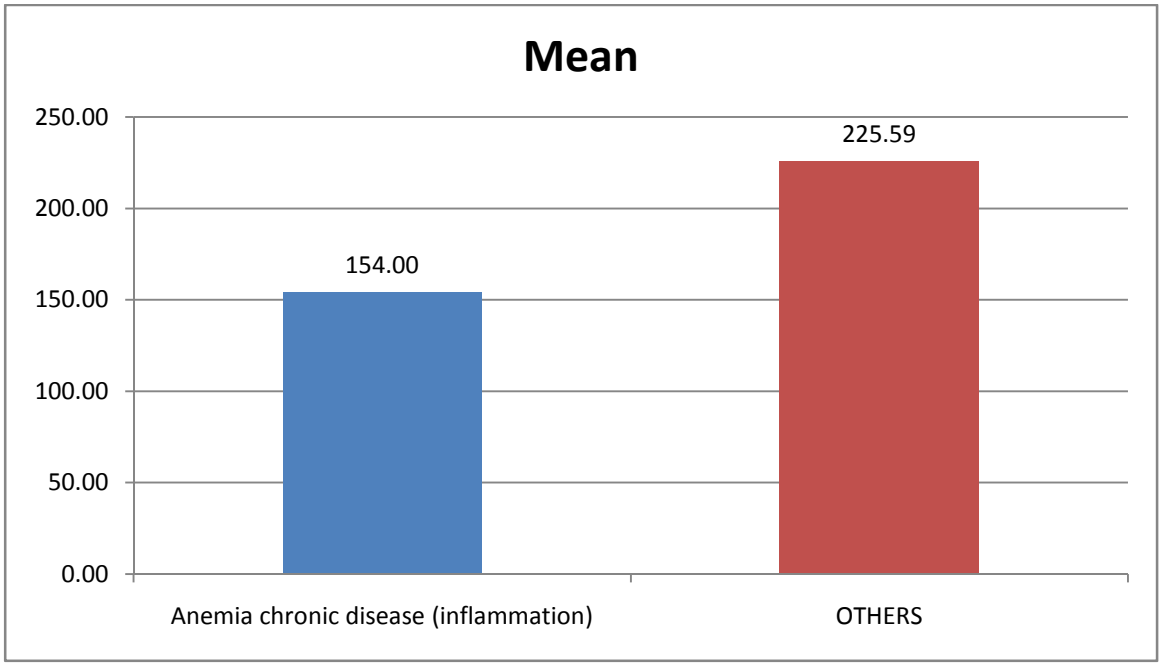


Table : 34 CD4 COUNT AND FUNCTIONAL CLASSIFICATION OF ANEMIA

CD4 COUNTS ETIOLOGY FUNCTION Cross tabulation			ETIOLOGY FUNCTION			Total
			Hypoproliferative Anemia	Maturation defect	Hemolysis	
CD4 COUNTS	<200	Count	27	7	1	35
		% within Etiology_Function	60.0%	53.8%	50.0%	58.3%
	>200	Count	18	6	1	25
		% within Etiology_Function	40.0%	46.2%	50.0%	41.7%
Total		Count	45	13	2	60
		% within Etiology_Function	100.0%	100.0%	100.0%	100.0%

Chart : 27

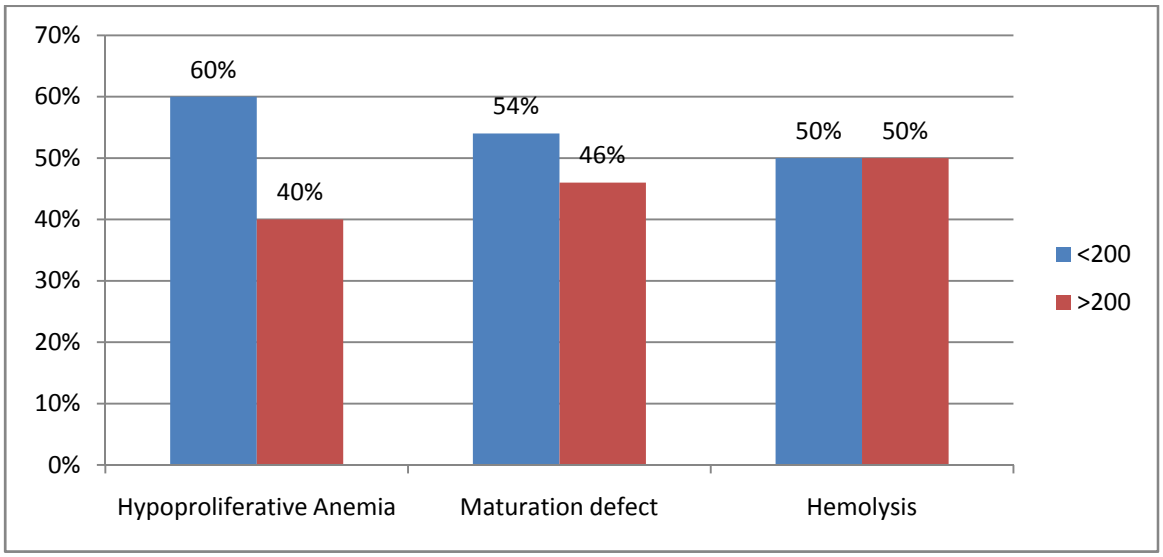
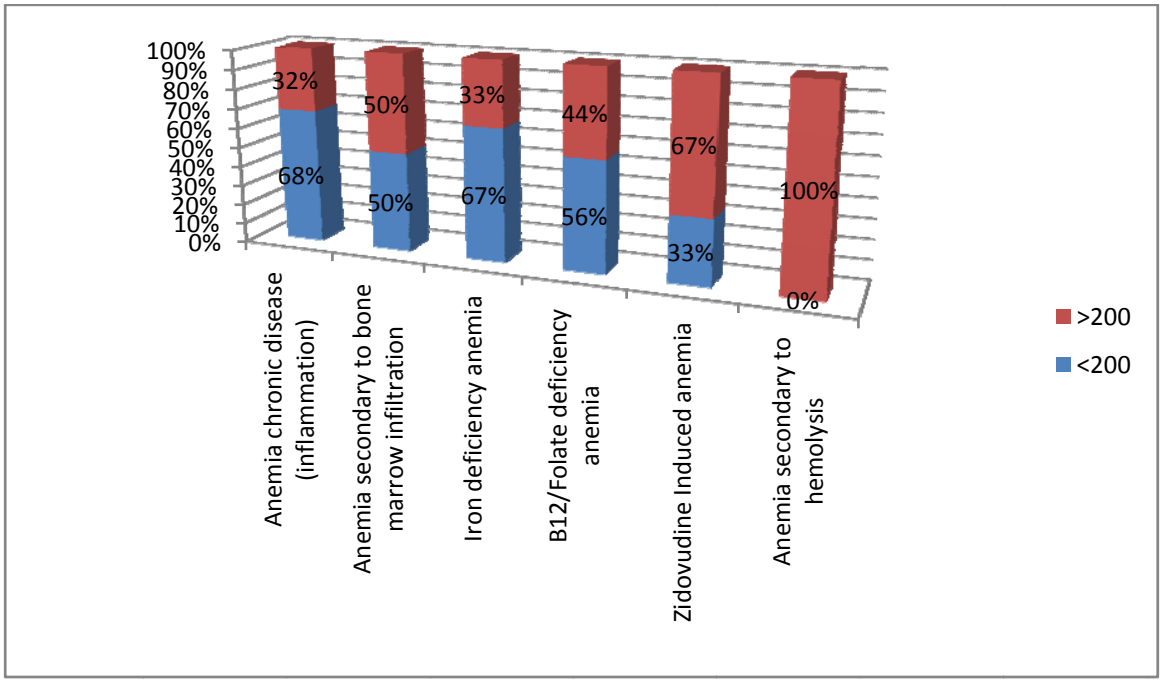


Table : 35 CD4 COUNTS AND VARIOUS ANEMIA CORRELATION

CD4 COUNTS * ETIOLOGY Crosstabulation			ETIOLOGY						Total
			Anemia chronic disease (inflammation)	Anemia secondary to bone marrow infiltration	Iron deficiency anemia	B12/Folate deficiency anemia	Zidovudine Induced anemia	Anemia secondary to hemolysis	
CD4 COUNTS	<200	Count	21	3	4	5	2	0	35
		%	67.7%	50.0%	66.7%	55.6%	33.3%	0.0%	58.3%
	>200	Count	10	3	2	4	4	2	25
		%	32.3%	50.0%	33.3%	44.4%	66.7%	100.0%	41.7%
Total		Count	31	6	6	9	6	2	60
		%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0 %

Chart : 28


100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
32%
68%
50%
50%
33%
67%
44%
56%
67%
33%
100%
0%
Anemia chronic disease (inflammation)
Anemia secondary to bone marrow infiltration
Iron deficiency anemia
B12/Folate deficiency anemia
Zidovudine Induced anemia
Anemia secondary to hemolysis
>200
<200

1. INTRODUCTION

6. DISCUSSION

In this study 60 consecutive patients infected with HIV (42 males and 18 females) and anemia who were admitted to the medical wards were included. Patients with mild anemia (>10.9g/dl) were excluded from the study. Only patients with hemoglobin less than 10.9 gm/dl (more than grade one of WHO/ACTG criteria) were included in the study[29].

DEMOGRAPHY

Minimum age of the subjects was 22 years and the maximum age was 60 years (Mean ± SD: 41.23±8.52). 70% of the subjects were male and 30% were female. Mean age in males was 42.5(SD 8.8) years and in females was 39 (SD 8.0) years. The disease was seen affecting people in the most productive years of their live. These demographic data are similar to those documented in other studies done in India88. 88.3% percent of patients were married. The unmarried patients (11.7% of the total) were all males. Males have commonly acquired the disease through premarital and extramarital sexual contact, whereas females have mostly acquired the disease from their spouses. Females have generally been diagnosed as HIV positive during either routine or ante natal checkups or when their husbands came with opportunistic infections. Majority of our study population were from tamil nadu and adjoining part of Andhra Pradesh. Madras medical college is a major referral center for patients from these areas. Patients from Tamil Nadu and part of Andhra utilize the services of this hospital and the study can be considered as done in a South Indian population.

FEATURES OF PATIENTS WITH HIV AND ANEMIA

Among the patients in the study 81.3%had their diagnosis established within 1 year of this study and 18.7% had their diagnosis established greater than one year ago. This was much lesser compared to other standard studies[99]. The mean CD4 count was 193/µl. Mean CD4 count in males was 199/µl and in females was 200/µl. CD4 count was lesser than 200/µl in 58.3% of patients and was greater than 200/µl in 41.7% of the patients. These features were similar to the other studies done in South India. [99] Thus the patients included in this study had advanced disease. 60% percentage of the patients were on HAART. Among the patients on HAART 67% were on zidovudine and the remaining 33% were on stavudine along with lamivudine and an NNRTI.

There were no patients on any other NRTI. 75% of the total patients were on co-trimoxazole therapy.

Severity of anemia was classified according to ACTG/WHO grading. 16.7% had grade one, anemia, 31.7% had grade two,18.3% had grade threen and 33.3% had grade four anemia. This was in contrast to a study done by Owiredu et al[100] in an ART clinic in Ghana in which more patients were found to have grade one and two anemia in comparison to grade four anemia. (Grade one and two- 76%; grade three – 19%; grade four - 7%). This is probably because our study was done in a tertiary care hospital and majority of the patients had advanced disease and higher grades of anemia. Among our patients 53.3% had associated leucopenia and 11.7 % had associated thrombocytopenia. The prevalence of neutropenia ranges from 0.8% to 44% and thrombocytopenia ranges from 8% to 30% in patients with HIV according to other studies done by Zon et al and Murphy [77] et al [101] [102] The mean of mean corpuscular volume(MCV) was 89.2fl (Range 59-125fl). Seventy percent had their MCV in normal range. Sevnteen percent had MCV less than 76 fl and 13% had MCV greater than 96 fl. This is similar to the study done by Tripathi et al which showed normal MCV in 88.5 %, low MCV in 6.56 % and high MCV in 4.92 % of patients[32]. Sixty percent of patients with MCV less than 76 fl had iron deficiency anemia. Thus 40% of patients with low MCV had etiology of anemia other than iron deficiency & microcytosis cannot be taken as indicative of iron deficiency. Among the patients with macrocytosis 87.5% had B12/folate deficiency and 12.5% had zidovudine induced anemia. Macrocytosis is commonly attributed to NRTI therapy but in our study majority of these patients had a low levels of vitamin B12 or folic acid.

96.7%of patients had reticulocyte proliferation index (RPI) less than 2.5. The mean RPI was 0.71(Range 0.03- 4.40). This indicates that majority of these patients have hypoproliferative marrow due to various causes.

15 %of the patients had positive direct antiglobulin (Coomb's) test (DCT). However, only 3% had evidence of hemolysis (high lactate dehydrogenase levels; unconjagated hyperbilirubinemia). This finding is similar to the results of another study by Ellaurie et al which showed DCT positivity in 37% of HIV- persons but clinically not significant hemolysis [83]. This indicates that positive DCT in HIV

infection may simply be a results of polyclonal hypergammaglobulinemia which is common in HIV infection & may not necessarily mean hemolysis.[84 87 78].

FERRITIN AND B12 LEVELS

In our study 15% had low vitamin B12 levels. According to Paltiel et al laboratory findings in HIV sero positive individuals shows vitamin B12 decreased in up to 30% [74]. In a study by Matthew et al 13% had at least one low B12 level during the course of their infection [75]. In a study by Rule et al serum B12 in 218 asymptomatic HIV positive patients were significantly lower than of a HIV negative control group (P = 0.02). Falling CD4 counts were correlating with low vitamin B12 levels in this study. [76] However in our study there was not significant relation between vitamin B12 levels and CD4 count or Hb . In the study by Burkes et al done in HIV positive patients low B12 concentration was not correlating with macrocytosis and parenteral vitamin B12 replacement often failed to increase serum cobalamin levels. [77 78] In our study only 50% percentage of patients with low B12 levels had macrocytosis & absence of macrocytosis cannot be used to rule out B12 deficiency. While underabsorption is on &b off responsible for the low level cobalamin, in most patients the cause is notknown and may show a serum abnormality. This may be due to altered transcobalamin activity and further studies are being done to evaluate low vitamin B12 levels without hematological manifestations[77]

3.3%had low ferritin levels. In our study there was no related correlation between serum ferritin levels and CD4 count or hemoglobin . Even in the presence of absolute iron deficiency serum ferritin levels may be elevated in HIV infection because of chronic inflammation. Though 10% of patients in our study had absolute iron deficiency manifested by grade zero iron stores in the bone marrow only. 3.3%of patients had low serum ferritin levels. All patients with low ferritin levels had iron deficiency. Thus normal ferrtin level does not rule out iron deficiency in HIV positive patients as ferritin is known as acute phase reactant & gets increased in inflammatory states.

Most patients in our study had high serum ferritin levels. Riera et al reported a high prevalence of elevated serum and red cell ferritin levels in 168 patients with HIV infection. High serum ferritin levels were found to correlate with clinical worsening of infection and with decreasing CD4+ lymphocyte counts ($p < 0.001$) in this study.[103]

PERIPHERAL SMEAR AND BONE MARROW EXAMINATION

In peripheral smear examination, 78% of the patients had normocytic normochromic anemia. Microcytes were present in 22% and 20% had macrocytes. There was a minimal discrepancy between the values obtained on measurement of MCV & the impression of the pathologist on the peripheral smear. Anisopoikilocytosis was noted in 60%. Tear drop cells were seen in 40% of patients. In the study by Jam et al RBC morphology readed as macrocytosis in 11%, normocytosis in 41.1% and microcytosis in 47.9% of the patients.[104] Erythrocyte morphology is described as normal and also abnormalities such as anisocytosis poikilocytosis, rouleaux formation have been reported[2]

Bone marrow was particularly examined for cellularity, dysplasia, and fibrosis, infiltration by infectious agents or malignant cells and iron staining. Common histopathological features, suggestive of HIV infection but non-pathognomonic as reported in other studies were hypocellularity, dysplasia, and lymphocytic and histiocytic infiltrates with or without granulomas, reticular fibrosis and increased iron deposits. [105]

According to Hoffman et al dysplastic changes were seen in 30% patients in myeloid line and 28% showed dysplastic changes in erythroid line.[66] Dysplasia may manifest as megaloblastic change. In our study 87% had normocellular and 13% were found to have hypocellular marrow. 85% percent had normoblastic maturation and 15 % had megaloblastic maturation. 25% had dysplastic changes in bone marrow. Hypocellularity in bone marrow in HIV positive patients range from 6.75% according to Tripathi et al to 34% according to Xiaohui et al respectively. [32] [33] In our study among patients with hypocellular marrow 75% had CD4 count less than 200. As reported by Marche et al hypocellularity in bone marrow is usually associated with severe immunodeficiency[105]

ETIOLOGY OF ANEMIA

In terms of functional classification, 75% had hypoproliferative anemia. 21.7% had maturation defects. 3.3% had anemia secondary to hemolysis. The largest number of patients had anemia of chronic disease in comparison to other etiologies. 51.7% had anemia of chronic disease, 10% had anemia secondary to bone marrow infiltration, 10% had iron deficiency anemia, 15% had B12/folate deficiency, 10% had anemia secondary to use of zidovudine, and 3.3 % had anemia due to hemolysis. Among the patients who had bone marrow infiltration, 33.3% (n=2) had tuberculosis and 66.6 % (n=4) had lymphoma.

Among the four patients who had lymphoma one patient had high grade T cell lymphoma and three patients had low grade B cell lymphomas. The relative risk of developing lymphoma is high in people with AIDS. In a study by Kote et al, the incidence of lymphoma increased by 165 fold in people with AIDS within 3 years of diagnosis compared to people without AIDS.[56] The increase in risk ranged from 652-times for high-grade diffuse immunoblastic tumors to 14-times for low-grade lymphomas.[56] In our study all the patients with lymphoma had CD4 count < 200. Studies have documented a definite correlation between the CD4 count and incidence of lymphoma. The risk of NHL is greatly increased with low CD4 counts. According to Bhaskaran et al[106] the relative risk of NHL increased to 6.3 at CD4 cell counts < 200/µl in comparison to patients with CD4 count greater than 350/µl. This corresponds to the details in the EuroSIDA studies which state that there is a decline in incidence of lymphoma in the HAART era (with improvement in immune status) comparing to the pre-HAART era.[58] In our study in 2% of patients bone marrow examination revealed a diagnosis of tuberculosis. In a prospective study done by David et al [53] in 105 adults with HIV infection and anemia 33% of patients had tuberculosis. It was diagnosed only by bone marrow culture in 8% of patients. As TB is the 2nd common opportunistic diseases in India (next to oral candidiasis), it may greatly increase the burden of anemia in this population. This underscores the importance of bone marrow examination in the evaluation of anemia in these patients, especially in patients with CD4 count less than 200/µl [88].

CD4 COUNT AND ANEMIA

An attempt was made to look for any relationship between immunological status as indicated by CD4 count and anemia as indicated by hemoglobin levels. Many prospective and cross sectional studies have shown a good correlation between Hb and CD4 counts. In resource poor settings, hemoglobin could be used as a surrogate marker for CD4 count for monitoring of disease progression according to a study by Christian et al done in Ghana. This showed there was a significant and positive interpretation ($r2 = 0.1755$; $p < 0.0001$) between the blood hemoglobin level and their respective CD4 counts [107]. This association is most likely explained by fact that disease progression could be associated with cytokine mediated myelosuppression. However various factors not related to disease progression may interfere in the direct relationship between CD4 count and hemoglobin including antiretroviral therapy, blood loss etc. and need to be excluded as in the above study. There may be no correlation between hemoglobin and anemia if all the etiologies of anemia are included as in our study.

There was no relevant difference in Hb values among patients who had CD4 count < 200/µl (mean Hb 5.65gm/dl) and CD4 count greater than 200/µl (mean Hb 5.54gm/dl) ($p=0.892$). There was relation between CD4 count and hemoglobin ($r=0.089$, $p=0.02$) in our study. There was similar to the findings of a study done by Seyed et al in Iran which showed poor correlation ($R =0.451$, $P = 0.056$) [108] As there was lack of correlation between hemoglobin and CD4 count further statistical analysis was done to look for relation between CD4 count and etiology of anemia.

Mean CD4 (119/µl) in patients who had anemia of chronic disease is significantly lesser than mean CD4 (270/µl) in patients who had anemia due to other etiologies ($p<0.001$). Among patients with low immunological status as expressed by CD4 count less than 200/µl, the etiologies of anemia were anemia of chronic disease (51.7%), anemia secondary to bone marrow infiltration (10.0%), B12/folate deficiency and zidovudine induced anemia (25%). In patients with CD4 count greater than 200/µl, the most common etiologies of anemia were iron deficiency (26.1%), Vitamin B12/folate deficiency and zidovudine induced anemia (39.1%), anemia of chronic disease (26.1%) & anemia secondary to hemolysis (8.7%). There were no

cases of anemia secondary to bone marrow infiltration. All patients with bone marrow infiltration had CD4 count < 200/µl, and thus bone marrow examination was very important in patients with low CD4 count. In patients with CD4 count > 200/µl, anemia in chronic disease was less common and other etiologies were comparable to HIV negative patients.

CD4 COUNT AND OTHER FACTORS

In our study there was significant difference in the bone marrow iron staining among patients with low immunological status as expressed by CD4 count < 200/µl, in comparison with persons having CD4 count greater than 200/µl. Among patients with CD4 count < 200/µl, eighty six percentage of patients had more than normal marrow iron staining in macrophages (grade 4 to 5) whereas in patients with CD4 count > 200/µl, only fifty six percentage had high bone marrow iron stores. (p=0.002). This is similar to other studies which show that increased bone marrow Fe stores are found in patients with severe disease. In a study by Cecile et al[81], three hundred and forty-eight HIV positive patients were analyzed for the involvemant of Fe stores on survival. Patients with grades 4 to 5 Fe stores (increased) were compared to those with grades 0–2 Fe stores (normal). In this study there was severe immunodeficiency in patients with high macrophage iron .[81] A large study from [84] Gambia found that increased iron status was associated with increased mortality in HIV positive patients. [109] Iron supplementation in HIV positive patients without evidence of true iron deficiency may be harmful since excessive iron accumulation is associated with enhanced oxidative stress and progression of HIV infection [82]

DRUGS AND ANEMIA

According to Moinuddin et a[160] > 20% of cases of anemia associated with HIV are drug causing. Recognizing this like specific side effect is usually problematic by the fact that progressive disease, opportunistic infections & bone marrow infiltration can all cause anaemia in these patients, in addition to other usual causes seen in non-HIV infected patients. Additionally, many patients are on multiple drugs and to determine the effect of individual drugs can be difficult. Most prospective studies in Europe and North America have reported that ART is

associated with resolution of HIV-associated anemia. [70] [110] [111]. A study from rural Uganda found that the mean Hb increased from 11.3 g/dL at baseline to 12.8 g/dL after twelve months on ART.[112].

In our study mean hemoglobin of patients on HAART (5.58 gm/dl) was found to be low comparing to people not on HAART (7.52 gm/dl). (P<0.001). However, this cannot be interpreted as HAART causing anemia because there has been a pre-selelection to include only patients with grade I -IV anaemia, patients on HAART would obviously have had more advanced disease, the numbers were too small to correlate the hemoglobin levels with the duration of HAART & patients were not longitudinally followed up as this was a cross-sectional study. It is well documented that other than when there is drug induced anaemia, HAART is associated with progressive improvement in anaemia.

There was significant difference in the hemoglobin levels in patients on zidovudine (5.54gm/dl) and non-zidovudine regimens (6.98 gm/dl) . 27% of patients on zidovudine had zidovudine induced anemia. In a prospective study done in Varanasi India in which patients were followed up over a period of one year 16.2 patients developed ZDV induced anemia[63]. In most patients, anemia developed within six months of start of therapy as seen in other studies. [66] In most of the patients (77%), there was a sharp recovery in hemoglobin levels after stopping zidovudine within a month. Peripheral smear examination in this study showed normocytic, normochromic picture in 42 per cent patients and others showed macrocytic changes. In our study 37.5% had normocytic, normochromic picture and others showed macrocytic changes.

In our study, the mean mean corpuscular volume was significantly higher in patients on ART (91.9 fl) compared to patients not on ART (84.2 fl). There is no significant difference in the mean MCV in patients on zidovudine (92.11fl) & non-zidovudine (91.51fl) regimens. (P=0.922). In a study by Moyle et al both AZT and d4T induce macrocytosis.[29] Macrocytosis can be expected in patients on either of these two drugs.

ANEMIA AND OTHER FACTORS

In our study, the mean hemoglobin was significantly higher in newly diagnosed patients (7.34 gm/dl) compared to patients who had been diagnosed earlier (6.44 gm/dl). Many of the newly diagnosed patients were probably in a less advanced state of the disease. Drug induced anaemia could have also contribute to this. It could also be artifactual as only patients with mild, moderate or more severe anaemia have been included .

There was no association between hemoglobin and gender. There was no relevant difference in the mean hemoglobin levels among various functional causes of anemia. The mean hemoglobin levels did not significantly differ among various etiologies of anemia.

LIMTATIONS OF THE STUDY

- The study has a few limitations that needs to be mentioned
- The number of cases studied was 60. This number is much smaller than the numbers studied in many major studies
- The study was a cross sectional one with minimal follow up due to time constraints. This did affect serial monitoring of patients.
- There is a selection bias. A greater proportion of patients with low CD4 counts have been included in the study as it has been done in a tertiary care center.

7. CONCLUSION

In this study, sixty patients with HIV infection and anemia were studied to find the etiology of anemia in HIV infected patients and find the relationship between anemia and immunological status as mention by the CD4 count. Only patients with Hb less than 10.9 g/dl (more than grade I of WHO/ACTG criteria) were included in this study.

Mean age of the patients was 40.97 years and the disease was seen affecting people in the most productive years of their life. Majority of the subjects were men (73%).

CD4 count was lesser than 200/µl in 58% of patients and was greater than 200/µl in 42% of the patients. Thus the patients included in this study had advanced disease. Mean CD4 count in males was 199/µl and in females were 200/µl.

Sixty percent of the patients were on HAART. Among the patients on HAART 67% were on zidovudine and the remaining 33%were on stavudine along with lamivudine and an NNRTI.

16.7% had grade oneanemia,31.7% had grade two anemia, 18.3% had grade three and 33.3% had grade four anemia. This is probably because our study was done in a tertiary care hospital and majority of the patients had advanced disease.

Fifty three percent had associated leucopenia and 11.7% had associated thrombocytopenia.

Seventy percent had their MCV in normal range. Seventeen percent had MCV less than 76 fl and 13% had MCV greater than 96 fl. On peripheral smear, 68.3% of the patients had normocytic normochromic anemia. Microcytes were present in 13.3% and 13.4% had macrocytes. Peripheral smear is more sensitive than MCV in the evaluation of anaemia.

Sixty percent of patients with MCV less than 76 fl had iron deficiency anemia. Forty percent of patients with low MCV had etiology of anemia other than iron deficiency. Among the patients with macrocytosis 87.5% had B12/folate deficiency and 12.5% had zidovudine induced anemia.

Ninety seven percent of the patients had reticulocyte proliferation index (RPI) less than 2.5. This indicates that majority of these patients have hypoproliferative marrow due to various causes.

Fifteen pecent of the patients had positive direct antiglobulin (Coomb's) test (DCT). However, only 3% had evidence of hemolysis. This indicates that positive DCT in HIV infection may not necessarily mean hemolysis.

Thirteen percent had low vitamin B12 levels. However only 50% percent of patients with low B12 levels had macrocytosis.

Ten percent of patients in our study had absolute iron deficiency , however only half of these patients had low serum ferritin levels. Thus a normal ferritin level does not rule out iron deficiency in HIV positive patients.

In bone marrow examination, 87% had normocellular and 13% were found to have hypocellular marrow. Eighty five percent had normoblastic maturation and 15 % had megaloblastic maturation. 25% had dysplastic changes in bone marrow.

Among our patients with hypocellular marrow 75% had CD4 count less than 200/µl.

The largest number of patients had anemia of chronic disease in comparison to other etiologies. Fifty two percent had anemia of chronic disease, 15% had B12/folate deficiency, 10% had bone marrow infiltration, 10% had iron deficiency anemia, 10% had zidovudine induced anemia and 3% had anemia due to hemolysis.

Among the patients who had bone marrow infiltration (n = 6), 33.3% (n =2) had tuberculosis and 66.66% (n = 4) had lymphoma. All the patients with lymphoma had CD4 count less than 200/µl. In one of the patients who had tuberculous infiltration of the marrow, this was the only evidence of tuberculosis

There was no relevant difference in Hb values among patients who had CD4 count < 200/µl (mean Hb 5.65gm/dl) and CD4 count greater than 200/µl (mean Hb 5.54gm/dl) (p=0.897). There was no relation between CD4 count and hemoglobin (r=0.089, p=0.498).

However, mean CD4 count (119/µl) in patients who had anemia of chronic disease was significantly lesser than the mean CD4 count (270/µl) in patients who had anemia due to other etiologies ($p<0.001$).

Among patients with low immunological status as expressed by CD4 count less than 200/µl, the etiologies of anemia were anemia of chronic disease (67.6%), anemia secondary to bone marrow infiltration (16.2%) B12/folate deficiency and zidovudine induced anemia (16.2%). Among patients with CD4 count greater than 200/µl, the etiologies of anemia were iron deficiency (26.1%), Vitamin B12/folate deficiency and zidovudine induced anemia (39.1%), anemia of chronic disease (26.1%) & anemia secondary to hemolysis (8.7%). There were no cases of anemia secondary to bone marrow infiltration in patients with CD4 count > 200/µl.

Among patients with CD4 count less than 200/µl, 86% of patients had more than normal marrow iron staining whereas in patients with CD4 count > 200/µl, only 56% had high bone marrow iron stores. ($p=0.002$). This indicates that with increasing immunodeficiency anaemia of chronic disease becomes more common.

In our study mean hemoglobin of patients on HAART (5.58 gm/dl) was found to be low comparing to people not on HAART (7.52 gm/dl). ($P<0.001$). This could be because patients on HAART would obviously have had more advanced disease.

There was significant difference in the hemoglobin levels in patients on zidovudine (5.54gm/dl) and non-zidovudine regimens (6.98 gm/dl) ($P=0.011$). Twenty seven percent of patients on zidovudine had zidovudine induced anemia. Among patients with zidovudine induced anemia, 37.5% had normocytic, normochromic picture and others showed macrocytic changes.

The mean mean corpuscular volume was significantly higher in patients on ART (91.9 fl) compared to patients not on ART (84.2 fl). ($P=0.030$) There was no relevant difference in the mean MCV in patients on zidovudine (92.11fl) & those not on zidovudine (91.51fl).

The mean hemoglobin was significantly higher in newly diagnosed patients (7.34 gm/dl) compared to patients who had been diagnosed earlier. This could signify an earlier stage of disease in newly diagnosed individuals.

8. SUMMARY

HIV positive patients have anemia is increasing in frequency that is parallel to the increasing number of new cases seen world over. The World Health Organization (WHO) estimates that around 36.7 million peoples in the world are infected with HIV. Approximately 95% of these cases as well as deaths from AIDS occur in the developing world. India has the 2nd largest burden of HIV related pathology, after Sub-Saharan Africa. Of the subtypes of HIV virus, subtype C is responsible for more than 50% of HIV 1 infections causing rapidly growing epidemics in India.

HIV positive patients maximum incidence of anemia will ranges from 10% in asymptomatic patients to 92% in individuals with full blown AIDS. In HIV positive patients anemia is a prognostic marker for future disease progression or death, not dependent of CD4 count and viral load. Though anemia directly does not contribute to mortality, it causes significant morbidity. Anemia impacts a range of dimensions of quality of life. The common causes of anemia in Humanimmunodeficiency and non HIV patients are different & treatment will differ. It may require the modification of antiretroviral drugs and the drugs used for the treatment of opportunistic infections. Hence knowledge of the pathophysiological mechanisms and the prevalence of various causes of anemia will help us in treatment of anemia in HIV positive patients.

Very few studies have studied the etiology of anemia in these patients in developing countries. The objective of our study was to study the etiology of anemia in HIV positive patients and to study the relationship between anemia and immunological status as indicated by the CD4 count. This was a descriptive cross sectional study conducted in Medical wards in MADRAS MEDICAL COLLEGE,CHENNAI.

Sixty HIV positive patients with HIV and anemia (Grade I or greater of WHO/ACTG criteria) were analyzed. Mean age was 40.97 years. Seventy three percent of the subjects were male. Among the patients in the study, 76.6% had their diagnosis established within 1 year of this study and 23.3% had their diagnosis established greater than one year ago. CD4 count was lesser than 200/µl in 58% of patients. Sixty percentage of the patients were on HAART. 16.7% grade one,

31.7% percent had grade two anemia, 18.3%% had grade three and 38.3% had grade four anemia. Seventeen percent had MCV less than 76 fl and 13% had MCV greater than 96 fl. Sixty percent of patients with MCV less than 76 fl had iron deficiency anemia. Microcytosis cannot be taken as indicative of iron deficiency. Among the patients with macrocytosis 87.5% had B12/folate deficiency and 12.5% had zidovudine induced anemia. Fifteen pecent of the patients had positive direct antiglobulin (Coomb's) test (DCT). However, only 3% had evidence of hemolysis. 13% had low vitamin B12 levels. In our study only 50% percent of patients with low B12 levels had macrocytosis. Though 10% of patients in our study had absolute iron deficiency only 5% of patients had low serum ferritin levels. In peripheral smear examination, 78% of the patients had normocytic normochromic anemia. Eighty seven percent had normocellular and 13% were found to have hypocellular marrow.

The largest number of patients had anemia of chronic disease in comparison to other etiologies. Fifty two percent had anemia of chronic disease, 15% had B12/folate deficiency, 10% had bone marrow infiltration, 10% had iron deficiency anemia, 10% had zidovudine induced anemia and 3% had anemia due to hemolysis.

Among the patients who had bone marrow infiltration (n = 6), 33.3 % had tuberculosis and 66.6 % had lymphoma. All the patients with lymphoma had CD4 count less than 200/µl. This underscores the significant of bone marrow examination in the evaluation of anemia in these patients especially in patients with CD4 counts < 200/µl.

There was significant difference in hemoglobin values among patients who had CD4 count < 200/µl and CD4 count greater than 200/µl. In patients with CD4 count > 200/µl, anemia of chronic disease was less common and other etiologies were comparable to HIV negative patients. 27% of patients on zidovudine had zidovudine induced anemia.

The mean corpuscular volume was significantly higher in patients on ART compared to patients not on ART. There was no relevant difference in the MCV in patients on zidovudine and those not on zidovudine..

The etiology of anemia is different in HIV positive patients, especially in patients with CD4 count less than 200/µl comparing to HIV negative patients. Evaluation of anemia should focus on establishing a specific etiology as the therapeutic options differ based on the etiology.

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10. ANNEXURE

PROFORMA

Name Ht/Wt

Age

Sex

Occupation

Address

Marital status

Duration since HIV diagnosis

CD4 count

Treatment details

- HAART

(1)ZDV/non ZDV containing regimen –

Duration of treatment

- Anti opportunistic treatment

Co trimoxazole

Dapsone

Gancyclovir

Total count

Hemoglobin

Reticulocyte proliferation index

MCV

Peripheral smear

Dct

Vitamin B12 levels

Serum iron

Serum ferritin

If needed investigations :

- Bone marrow Aspiration
  - Presence or absence of erythropoisis
  - Cellularity
  - Type of maturation
  - Iron stores
  - Reticulin in the bone marrow
- Biopsy
  - Infiltration by lymphoma/fungus/granuloma
- Stool routine
  - Microscopy
  - Occult blood
- Sputum AFB
- FNAC

LN biopsy

Abdomen USG

LFT

Clinical features

Presence/absence of PUO

Predominant system involved

Final diagnosis

Presence of clinical AIDS defining illness

LIST OF ABBREVIATIONS USED

ACTG AIDS	Clinical Trial Group
AIDS	Acquired immune deficiency syndrome
ART	Anti retroviral therapy
AZT	Zidovudine
CCR5	C-C chemokine receptor type 5
CD	Cluster of differentiation
CDC	Centre for disease control
CXRCR5	C-X-C chemokine receptor type 5
DCT	Direct coobs test
DNA	Deoxy ribo nucleic acid
D4T	Stavudine
EURO SIDA	European Swedish International Development Co-operation Agency
ELISA	Enzyme linked immunosorbent assay
FDA	Food and drug administration
Fe	Iron
G- CSF	Granulocyte colony stimulating factor
HAART	Highly active anti retroviral therapy.
HBV	Hepatitis B virus
Hb	Hemoglobin
HCV	Hepatitis C virus
HIV	Human Immunodeficiency virus
IL	Interleukin
KS	Kaposi Sarcoma
MCV	Mean corpuscular volume.
MAI	Mycobacterium avium intracellulare

MHC	Major Histocompatibility complex
NACO	National AIDS control organization. VIII
OI	Opportunistic infections
NHL	Non hodgkins lymphoma
NRTI	Nucleoside reverse transcriptase inhibitors.
PCP	Pneumocystis jiroveci pneumonia
WHO	World Health Organisation.
3TC	Lamivudine
ZDV	Zidovudine IX

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI 600 003

EC Reg.No.ECR/270/Inst./TN/2013
Telephone No.044 25305301
Fax: 011 25363970

CERTIFICATE OF APPROVAL

To
Dr.Senthil.A.
Post Graduate in M.D. (General Medicine)
Institute of Internal Medicine
Madras Medical College
Chennai 600 003

Dear Dr.Senthil.A,

The Institutional Ethics Committee has considered your request and approved your study titled **"A STUDY OF FACTORS ASSOCIATED WITH ANEMIA IN HIV INFECTED INDIVIDUALS IN A TERTIARY CARE HOSPITAL"** - **NO.(II) 06032016.**

The following members of Ethics Committee were present in the meeting hold on **22.03.2016** conducted at Madras Medical College, Chennai 3

1.Dr.C.Rajendran, MD.,	:Chairperson
2.Dr.R.Vimala,MD.,Dean,MMC,Ch-3	:Deputy Chairperson
3.Prof.Sudha Seshayyan,MD., Vice Principal,MMC,Ch-3	: Member Secretary
4.Prof.P.Raghumani,MS, Dept.of Surgery,RGGGH,Ch-3	: Member
5.Dr.Baby Vasumathi, Director, Inst. of O&G,Ch-8	: Member
6.Prof.M.Saraswathi,MD.,Director, Inst.of Path,MMC,Ch-3	: Member
7.Prof.Srinivasagalu,Director,Inst.of Int.Med.,MMC,Ch-3	: Member
8.Tmt.J.Rajalakshmi, JAO,MMC, Ch-3	: Lay Person
9.Thiru S.Govindasamy, BA.,BL,High Court,Chennai	: Lawyer
10.Tmt.Arnold Saulina, MA.,MSW.,	:Social Scientist

We approve the proposal to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

MADRAS MEDICAL COLLEGE
31 MAR 2016
CHENNAI-600 003

Member Secretary – Ethics Committee

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE
CHENNAI-600 003

INFORMATION SHEET

We are conducting a study on **"A STUDY OF FACTORS ASSOCIATED WITH ANEMIA IN HIV INFECTED INDIVIDUALS IN A TERTIARY CARE HOSPITAL"** among patients attending Rajiv Gandhi Government General Hospital, Chennai and for that your specimen may bevaluable to us.

The purpose of this study is to find the factors a associated with anemia in HIV patients in tertiary care hospital. In HIV patients admitted in medical ward, clinical history of patients are taken, then laboratory investigations will be done after their consent, blood collected for blood haemoglobin, ferritin, CBC, peripheral smear, serum ferritin and B12 level, DCT, Bone marrow aspiration and other necessary investigations are taken to find cause of anemia.

The privacy of the patients in the research will be maintained throughout the study. In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared.

Taking part in this study is voluntary. You are free to decide whether to participate in this study or to withdraw at any time; your decision will not result in any loss of benefits to which you are otherwise entitled.

The results of the special study may be intimated to you at the end of the study period or during the study if anything is found abnormal which may aid in the management or treatment.

Signature of Investigator **Signature of Participant**

Date :

Place :

ஆராய்ச்சியில் பங்கேற்பவர்கான தகவல் அறிக்கை

ஆராய்ச்சியின் தலைப்பு : உயர் சிகிச்சை மருத்துவ மனையில் உள் நோயாளிகளாக அனுமதிக்கப்பட்டுள்ள எச்.ஐ.வி. நோயுடையோரின் இரத்த சோகைக்கான காரணிகளைப்பற்றிய ஆய்வு.

பங்குகொள்பவரின் பெயர் :

ஆராய்ச்சி செய்பவரின் பெயர் : செந்தில். ஆ

இடம் : ராஜீவ் காந்தி அரசு பொது மருத்துவமனை

சென்னை – 600 003.

இந்த ஆராய்ச்சி / ஆய்வு / செய்முறை / சோதனையில் தாங்கள் பங்கேற்க அழைக்கிறோம். இந்த தகவல் அறிக்கையில் கூறப்பட்டிருக்கும் தகவல்கள் தாங்கள் இந்த ஆராய்ச்சியில் பங்கேற்கலாமா, வேண்டாமா என்பதை முடிவு செய்ய உதவியாக இருக்கும். இந்த படிவத்தில் உள்ள தகவல்கள் பற்றி உள்ள சந்தேகங்களை நீங்கள் தயங்காமல் கேட்கலாம்.

இந்த ஆய்வின் நோக்கம் என்ன ?

எச்.ஐ.வி. நோயால் பாதிக்கப்பட்டு உள்நோயாளிகளாக இருக்கும் நோயாளிகளின் இரத்த சோகைக்கான காரணிகளை கண்டறிதல்.

ஆய்வு முறைகள் :

விரிவான நோய்க் குறிப்புகளும், மருத்துவ பரிசோதனைகளும் செய்யப்படும். நோயாளிகள், அவர்கள் சம்மதத்திற்கு பின் குருதியில் ஹீமோகுளோபின், பெரிபரல் ஸ்மியர், ரெட்டிகுலோசைட் கவுண்ட், இரத்த அணுக்களின் கன அளவு, பெரிட்டின் அளவுகள், பி12 அளவு, டைரக்ட் கூம்ப்ஸ் டெஸ்ட், தேவைப்பட்டால் எலும்பு சாறு பரிசோதித்தல் மற்றும்

நோயாளிகளுக்கு ஏற்றவாறு தேவைப்படும் மற்ற பரிசோதித்து இரத்த சோகையின் காரணிகளை கண்டறிதல்.

ஆய்வினால் மக்களுக்கு ஏற்படும் நன்மைகள் :

இந்த ஆய்வின் முடிவில் கிடைக்கும் தகவல்கள் சமுதாயத்திற்கு பயனுள்ளதாகவும், எதிர் காலத்தில் நோயாளிகளுக்கு மருத்துவ தீர்வாகவும் அமையும்.

தங்களிடமிருந்து பெறப்படும் தகவல்களின் நம்பிக்கை தன்மை :

தங்களிடமிருந்து பெறப்படும் தகவல்கள் பாதுகாக்கப்படுவதற்கான முழு உரிமையும் தங்களுக்கு உண்டு.

PATIENT CONSENT FORM

Study Detail : "**A STUDY OF FACTORS ASSOCIATED WITH ANEMIA IN HIV INFECTED INDIVIDUALS IN A TERTIARY CARE HOSPITAL"**

Study Centre : Rajiv Gandhi Government General Hospital, Chennai.

Patient's Name :

Patient's Age :

In Patient Number :

Patient may check (☑) these boxes

I confirm that I have understood the purpose of procedure for the above study. I have the opportunity to ask question and all my questions and doubts have been answered to my complete satisfaction. ❑

I understand that my participation in the study is voluntary and that I am free to withdraw at any time without giving reason, without my legal rights being affected. ❑

I understand that sponsor of the clinical study, others working on the sponsor's behalf, the ethical committee and the regulatory authorities will not need my permission to look at my health records, both in respect of current study and any further research that may be conducted in relation to it, even if I withdraw from the study I agree to this access. However, I understand that my identity will not be revealed in any information released to third parties or published, unless as required under the law. I agree not to restrict the use of any data or results that arise from this study.

❑

I agree to take part in the above study and to comply with the instructions given during the study and faithfully cooperate with the study team and to immediately inform the study staff if I suffer from any deterioration in my health or well being or any unexpected or unusual symptoms. ❑

I hereby consent to participate in this study ❑

I hereby give permission to undergo complete clinical examination and diagnostic tests including hematological, biochemical, radiological tests. ❑

Signature/thumb impression

Patient's Name and Address:

Signature of Investigator

Study Investigator's Name:

Dr. SENTHIL A

ஆராய்ச்சி ஒப்புதல் படிவம்

ஆராய்ச்சியின் தலைப்பு: உயர் சிகிச்சை மருத்துவ மனையில் உள் நோயாளியாக அனுமதிக்கப்பட்டுள்ள எச்.ஐ.வி. நோயுடையோரின் இரத்த சோகைக்கான காரணிகளைப்பற்றிய ஆய்வு.

ஆராய்ச்சி செய்பவரின் பெயர் : செந்தில். ஆ

ஆராய்ச்சி மையம் : ராஜீவ் காந்தி அரசு பொது மருத்தவ மனை, சென்னை–600 003.

..... எனும் நான் எனக்கு கொடுத்துள்ள தகவல் தாளை படித்து புரிந்து கொண்டேன். நான் பதினெட்டுவயதை கடந்துள்ளதால், என்னுடைய சுய நினைவுடனும், முழு சுதந்திரத்துடனும் இந்த ஆராய்ச்சியில் என்னை சேர்த்துக்கொள்ள சம்மதிக்கிறேன்.

1. நான் எனக்கு அளிக்கப்பட்ட ஒப்புதல் படிவத்தையும், தகவல்களையும் படித்து புரிந்து கொண்டேன்.
2. ஒப்புதல் படிவத்தில் உள்ள தகவல்கள் எனக்கு விளக்கி கூறப்பட்டன.
3. ஆய்வின் தன்மை பற்றி எனக்கு விளக்கப்பட்டது.
4. என்னுடைய உரிமைகளையும், பொறுப்புகளையும் ஆராய்ச்சியாளர் விளக்கிக் கூறினார்.
5. நான் இதுவரை எடுத்துள்ள / எடுத் கொண்டிருக்கும் அனைத்து விதமான சிகிச்சை முறைகளையும் ஆராய்ச்சியாளரிடம் கூறியுள்ளேன்.
6. இந்த ஆராய்ச்சியினால் ஏற்படும் தீமைகள் பற்றி விளக்கப்பட்டன.

இடம் :

நாள் : கையொப்பம்

MASTER CHART

NAMES	AGE	SEX	IP NO	MARITAL STATUS	HIV ELISA	DRUGS	DURATION OF DISEASES	CD4 COUNTS	ART	Hb%	MCV	T C	PLATELETS COUNT	R P I %	S.B12	P S	DCT	S.FERRITIN
SEKAR	28	MALE	83917	UNMARRIED	POSITIVE	NO	< ONE MONTH	> 200	N	11	90	>4000	> ONE LAKSH	< 2.5	N	NNA	P	> 24
MUTHAIYAN	40	MALE	84018	MARRIED	POSITIVE	YES	EIGHT MONTHS	< 200	Y	8.5	79	< 4000	> ONE LAKSH	< 2.5	N	NNA	N	> 24
SRINIVASAN	45	MALE	108800	MARRIED	POSITIVE	YES	SEVEN MONTHS	< 200	Y	9.2	85	< 4000	> ONE LAKSH	< 2.5	N	NNA	N	> 24
YUVARAJ	29	MALE	114469	UNMARRIED	POSITIVE	NO	< ONE MONTH	> 200	N	10	< 76	> 4000	> ONE LAKSH	< 2.5	N	NNA	N	> 24
SANJEEV KUMAR	44	MALE	60460	MARRIED	POSITIVE	YES	TEN MONTHS	< 200	Y	9.3	94	< 4000	> ONE LAKSH	< 2.5	N	NNA	P	> 24
MUNUSAMY	52	MALE	117437	MARRIED	POSITIVE	YES	SIX MONTHS	< 200	Y	7.5	> 96	< 4000	< ONE LAKSH	< 2.5	L	Mg A	N	> 24
ARUNACHALAM	42	MALE	120070	MARRIED	POSITIVE	YES	EIGHT MONTHS	< 200	Y	8.1	< 76	< 4000	> ONE LAKSH	< 2.5	N	NNA	N	> 24
RAGUPATHY	51	MALE	66124	MARRIED	POSITIVE	YES	FIVE MONTHS	< 200	Y	7.1	> 96	< 4000	> ONE LAKSH	< 2.5	L	Mg A	N	> 24
VENKATESAN	32	MALE	134575	MARRIED	POSITIVE	NO	< ONE MONTH	> 200	N	9.7	92	> 4000	> ONE LAKSH	< 2.5	N	NNA	N	> 24
ABDUL SATHAR	52	MALE	91852	MARRIED	POSITIVE	YES	ELEVEN MONTHS	< 200	Y	< 6.5	79	< 4000	> ONE LAKSH	< 2.5	N	NNA	P	> 24
SRINVASAN	35	MALE	19495	MARRIED	POSITIVE	YES	< ONE MONTH	> 200	N	9.2	82	> 4000	> ONE LAKSH	< 2.5	N	NNA	N	> 24
SENTHIL KUMAR	36	MALE	24924	MARRIED	POSITIVE	YES	FOUR MONTHS	< 200	Y	9.3	85	< 4000	> ONE LAKSH	< 2.5	N	NNA	N	> 24
SURESH	40	MALE	2408	MARRIED	POSITIVE	YES	> TWELVE MONTHS	< 200	Y	7.8	< 76	< 4000	> ONE LAKSH	< 2.5	N	NNA	N	> 24
SUDHAKAR	30	MALE	3391	UNMARRIED	POSITVE	NO	< ONE MONTH	> 200	N	10	94	> 4000	> ONE LAKSH	< 2.5	N	MNA	N	> 24
MUNNA	48	MALE	10660	MARRIED	POSITIVE	YES	> TWELVE MONTHS	< 200	Y	8.5	> 96	< 4000	> ONE LAKSH	< 2.5	L	NNA	P	> 24
RAJENDRAN	50	MALE	13516	MARRIED	POSITIVE	YES	NINE MONTHS	< 200	Y	9.2	83	< 4000	> ONE LAKSH	< 2.5	N	NNA	N	> 24
KANNIYAPPAN	42	MALE	17128	MARRIED	POSITIVE	YES	> TWELVE MONTHS	< 200	Y	< 6.5	79	< 4000	< ONE LAKSH	< 2.5	N	NNA	N	> 24
VENUGOPAL	60	MALE	21775	MARRIED	POSITIVE	YES	SIX MONTHS	< 200	Y	< 6.5	> 96	< 4000	< ONE LAKSH	> 2.5	L	Mg A	N	> 24
SHANKAR	42	MALE	40940	MARRIED	POSITIVE	YES	> TWELVE MONTHS	< 200	Y	8.7	76	< 4000	> ONE LAKSH	< 2.5	N	MNA	N	> 24
SYED MEER SHEIK	44	MALE	62611	MARRIED	POSITIVE	YES	< ONE MONTH	> 200	Y	7.5	< 76	< 4000	> ONE LAKSH	< 2.5	N	NNA	N	> 24
ANANDHAN	42	MALE	62648	MARRIED	POSITIVE	YES	SEVEN MONTHS	< 200	Y	8.8	78	< 4000	> ONE LAKSH	< 2.5	N	MNA	N	> 24
SARAVANAN	27	MALE	65572	UNMARRIED	POSITIVE	NO	< ONE MONTH	> 200	N	11	94	> 4000	> ONE LAKSH	< 2.5	N	NNA	N	> 24
RAMESH KUMAR	48	MALE	68095	MARRIED	POSITIVE	YES	THREE MONTHS	> 200	Y	< 6.5	88	< 4000	> ONE LAKSH	< 2.5	N	MNA	N	> 24
PANNER SELVAM	48	MALE	73976	MARRIED	POSITIVE	YES	< ONE MONTH	< 200	Y	6.9	> 96	< 4000	> ONE LAKSH	< 2.5	L	Mg A	N	> 24
DEVAN	58	MALE	76903	MARRIED	POSITIVE	YES	> TWELVE MONTHS	< 200	Y	< 6.5	79	< 4000	< ONE LAKSH	< 2.5	N	NNA	N	> 24
PANDIYAN	45	MALE	90676	MARRIED	POSITIVE	YES	NINE MONTHS	< 200	Y	< 6.5	82	< 4000	> ONE LAKSH	< 2.5	N	MNA	N	> 24
SHANTHI	40	FEMALE	343108	MARRIED	POSITIVE	YES	TEN MONTHS	< 200	N	8.6	89	> 4000	> ONE LAKSH	< 2.5	N	NNA	P	> 24
SASIKALA	36	FEMALE	76354	MARRIED	POSITIVE	YES	TWO MONTHS	> 200	N	9.6	91	> 4000	> ONE LAKSH	< 2.5	N	MNA	N	> 24
CHELLAMMAL	50	FEMALE	90197	MARRIED	POSITIVE	YES	< ONE MONTH	< 200	Y	< 6.5	90	< 4000	> ONE LAKSH	< 2.5	N	MNA	N	> 24
SASIKALA	43	FEMALE	95719	MARRIED	POSITIVE	YES	EIGHT MONTHS	> 200	N	8	< 76	> 4000	> ONE LAKSH	< 2.5	N	NNA	P	> 24
DEVI	60	FEMALE	69934	MARRIED	POSITIVE	YES	> TWELVE MONTHS	< 200	Y	< 6.5	77	< 4000	< ONE LAKSH	> 2.5	N	NNA	N	< 24
THILAGAVATHY	32	FEMALE	94395	MARRIED	POSITIVE	YES	FIVE MONTHS	> 200	N	11	90	> 4000	> ONE LAKSH	< 2.5	N	NNA	N	> 24
MORJINA	30	FEMALE	72109	UNMARRIED	POSITIVE	NO	< ONE MONTH	> 200	N	9	95	> 4000	> ONE LAKSH	< 2.5	L	Mg A	N	> 24
CHENNAUER BARATI	45	FEMALE	88897	MARRIED	POSITIVE	YES	SIX MONTHS	< 200	N	7.4	< 76	> 4000	> ONE LAKSH	< 2.5	N	MNA	N	> 24
MEENA	30	FEMALE	23927	MARRIED	POSITIVE	YES	< ONE MONTH	< 200	Y	10	94	> 4000	> ONE LAKSH	< 2.5	N	NNA	N	> 24
RANJITHAM	45	FEMALE	29570	MARRIED	POSITIVE	NO	< ONE MONTH	> 200	N	7.8	85	> 4000	> ONE LAKSH	< 2.5	N	MNA	N	> 24
KANTHAKUMARI	37	FEMALE	33304	MARRIED	POSITIVE	YES	THREE MONTHS	< 200	N	8.7	91	> 4000	> ONE LAKSH	< 2.5	N	NNA	N	> 24
SHANTHI	35	FEMALE	60784	MARRIED	POSITIVE	YES	> TWELVE MONTHS	> 200	N	9.2	95	> 4000	> ONE LAKSH	< 2.5	N	NNA	N	> 24
GOMATHI	26	FEMALE	104849	UNMARRIED	POSITIVE	NO	< ONE MONTH	> 200	N	10	89	> 4000	> ONE LAKSH	< 2.5	N	NNA	N	> 24
JAYANTHI	22	FEMALE	100224	UNMARRIED	POSITIVE	NO	< ONE MONTH	> 200	N	9.1	86	> 4000	> ONE LAKSH	< 2.5	N	NNA	N	> 24
RANI	45	FEMALE	105634	MARRIED	POSITIVE	YES	ELEVEN MONTHS	< 200	Y	7.3	> 96	< 4000	> ONE LAKSH	< 2.5	L	Mg A	N	> 24
GOWRI	45	FEMALE	105805	MARRIED	POSITIVE	YES	> TWELVE MONTHS	> 200	N	< 6.5	78	> 4000	> ONE LAKSH	< 2.5	N	NNA	P	> 24
PANDIMEENA	37	FEMALE	72816	MARRIED	POSITIVE	YES	SEVEN MONTHS	< 200	Y	9.2	< 76	< 4000	> ONE LAKSH	< 2.5	N	NNA	N	> 24

RAJATHI	33	FEMALE	76884	MARRIED	POSITIVE	YES	NINE MONTHS	< 200	Y	8.9	87	> 4000	> ONE LAKSH	< 2.5	N	NNA	N	> 24
DEVAN	47	MALE	61953	MARRIED	POSITIVE	NO	< ONE MONTH	> 200	N	6.9	80	> 4000	> ONE LAKSH	< 2.5	N	NNA	N	> 24
SRINIVASALU	47	MALE	61953	MARRIED	POSITIVE	NO	< ONE MONTH	> 200	N	< 6.5	76	> 4000	> ONE LAKSH	< 2.5	N	MNA	P	> 24
RAMESH	43	MALE	87150	MARRIED	POSITIVE	YES	EIGHT MONTHS	< 200	Y	< 6.5	79	< 4000	> ONE LAKSH	< 2.5	N	NNA	N	> 24
JOSEPH	44	MALE	89783	MARRIED	POSITIVE	YES	> TWELVE MONTHS	< 200	Y	< 6.5	> 96	< 4000	> ONE LAKSH	< 2.5	L	Mg A	N	> 24
VIJAYARAGHAVAN	46	MALE	126315	MARRIED	POSITIVE	YES	SEVEN MONTHS	> 200	N	< 6.5	80	> 4000	> ONE LAKSH	< 2.5	N	MNA	N	> 24
EZHILKUMAR	40	MALE	12535	MARRIED	POSITIVE	YES	EIGHT MONTHS	< 200	Y	6.9	< 76	< 4000	> ONE LAKSH	< 2.5	N	NNA	N	> 24
RAVI	50	MALE	23699	MARRIED	POSITIVE	NO	< ONE MONTH	> 200	N	< 6.5	79	> 4000	> ONE LAKSH	< 2.5	N	NNA	N	> 24
SRINIVASAN	45	MALE	16890	MARRIED	POSITIVE	NO	< ONE MONTH	> 200	N	< 6.5	78	> 4000	> ONE LAKSH	< 2.5	N	NNA	N	> 24
MURUGAPANDIYAN	53	MALE	19771	MARRIED	POSITIVE	YES	ELEVEN MONTHS	< 200	Y	< 6.5	82	< 4000	< ONE LAKSH	< 2.5	N	NNA	N	> 24
SHEIK MALIK	27	MALE	22930	MARRIED	POSITIVE	NO	< ONE MONTH	> 200	N	9.5	80	> 4000	> ONE LAKSH	< 2.5	N	NNA	N	> 24
UDAYA KUMAR	42	MALE	28065	MARRIED	POSITIVE	YES	> TWELVE MONTHS	< 200	Y	< 6.5	< 76	< 4000	> ONE LAKSH	< 2.5	N	NNA	N	> 24
KARTHIKEYAN	32	MALE	91530	MARRIED	POSITIVE	NO	< ONE MONTH	> 200	N	9.4	94	> 4000	> ONE LAKSH	< 2.5	N	NNA	P	> 24
ANANDHAN	40	MALE	85234	MARRIED	POSITIVE	YES	SIX MONTHS	< 200	Y	< 6.5	92	< 4000	> ONE LAKSH	< 2.5	N	NNA	N	> 24
SELVARAJ	40	MALE	85866	MARRIED	POSITIVE	YES	TEN MONTHS	< 200	N	7.8	84	< 4000	> ONE LAKSH	< 2.5	N	NNA	N	< 24
NAGARAJ	35	MALE	83236	MARRIED	POSITIVE	YES	FIVE MONTHS	> 200	N	< 6.5	> 96	> 4000	> ONE LAKSH	< 2.5	L	Mg A	N	> 24
JAGAN	42	MALE	95394	MARRIED	POSITIVE	YES	> TWELVE MONTHS	< 200	Y	< 6.5	< 76	< 4000	< ONE LAKSH	< 2.5	N	NNA	N	> 24

KEY WORDS FOR MASTER CHART-

scular volume, TC- total count, RPI- Reticulocyte proliferation index, PS - Peripheral smear, DCT - Direct coomb's tes

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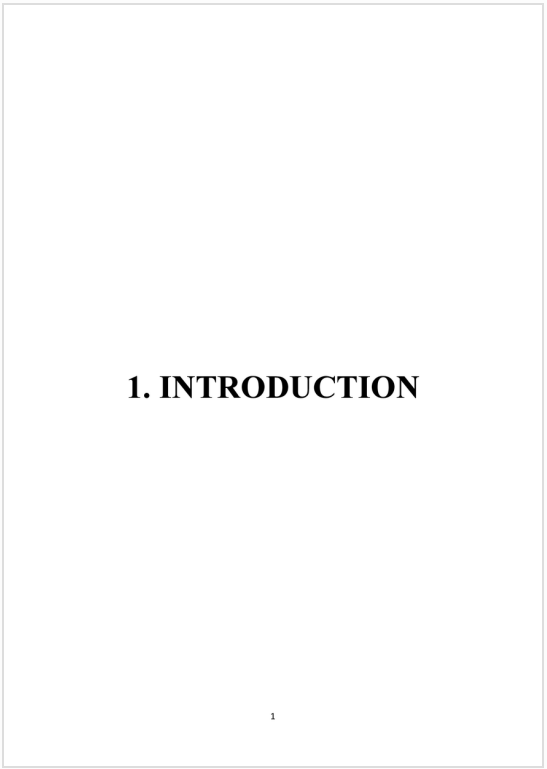

1. INTRODUCTION

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PLAGARISM REPORT